

Whose Rate for the Job?

THE British Government seems well on the way to making a nonsense of its labour relations with the scientists on its payroll. In the past few weeks, even those in the scientific civil service whose inclinations lie more in scholarly research than towards the corridors of power have been offended by the parsimony with which the Civil Service Department has dealt with the most recent investigation of the Pay Research Unit into the relationship between salaries in the scientific civil service and salaries of scientists elsewhere. Briefly, the members of the scientific civil service who are above the recruitment grades in status have been awarded no pay rise in the current year (see *Nature*, 232, 76; 1971). To be sure, many of those still working their way up the promotion ladders will have something extra in their pay packets, but there is little doubt that the settlement which the government has proposed, and which may well be modified by the compulsory arbitration to which it will now be subjected, represents a considerable injustice for many senior civil servants. One danger is that the bitterness evoked by this dispute will impede many of the reforms which have seemed to be in prospect in recent months as part of the painfully slow implementation of the Fulton report. More seriously, it is now possible that the government may wreck the whole basis on which it has proved possible in the past to persuade scientists to work in the public service.

What has gone wrong? To begin with, it is a great misfortune that the government's pay offer should be based on an apparently confidential piece of research carried out by the Pay Research Unit. Why not make this public? For objective comparisons between the salaries of civil servants, living as they do on a rigid salary structure, and scientists working in industry or commerce, are bound to be extremely hazardous. For one thing, the spread of salary in the civil service is much more closely linked with age than it is in industry. For another, no amount of arithmetic can accurately evaluate the fringe benefits which civil servants enjoy, especially holidays and pensions. After all, the importance of a lush but non-transferable pension scheme is that it persuades civil servants, especially the more senior of them, to put up with financial indignity rather than to move elsewhere.

The comparison between the civil service and industrial life is, however, most of all vitiated by the way in which promotion only rarely carries a scientist in the civil service into other kinds of activities. This was one of the complaints of the Fulton commission about the present organization of the civil service. Briefly, a man or woman who joins the scientific branch is likely to stay there until he or she retires. In other walks of life, and especially in industry, the scientific laboratories are increasingly (and rightly) regarded as stepping stones to administrative or managerial jobs. The result, of course, is that attempts to compare industry with the civil service are comparisons

of quite different populations. There may be something in the view, recently put forward by the Confederation of British Industries (see *Nature*, 230, 344; 1971), that the national interest would be better served if there were greater mobility between scientific research and administration, as indeed the Fulton commission has proclaimed. The fact that there are at present so few opportunities of this kind is not, however, the fault of the scientists concerned but of the government. It is entirely understandable that the scientists should feel hard done by.

However solemnly the advice of the Pay Research Unit may have been followed in the negotiations so far, the impression remains that the wellsprings of the government's meanness lie in its appraisal of future needs in the government research laboratories. With the continuing decline of defence research and with the doctrine of non-intervention as a guide, nobody will be surprised that the present British government should be more embarrassed than its predecessor by the numbers of scientists in the scientific civil service. It follows that in its pay negotiations, the government is bound to feel it has the whip hand. But is it really sensible to hope that a sufficient show of meanness will make that other problem go away by persuading young people not to work for the scientific civil service and by persuading more senior members to look elsewhere for new careers? Such arguments are certain to be recipes for continuing trouble. If the scientific civil service does have too many people, why not work out constructive ways of making fuller use of them?

The underlying question is the extent to which the British Government intends to make constructive use of scientists and their craft. So far, after more than a year in office, the government has had hardly anything to say on the subject. Although there is a sense in which, by comparison with the all too strident utterances of Mr Anthony Wedgwood Benn and Mr Harold Wilson on the subject, silence is a virtue, yet the lack of a policy in such an important field is indefensible. Moreover, there is no prospect that the government's problems will be solved at one fell swoop when Mrs Margaret Thatcher, the minister for education and science, has had a chance to mull over Sir Frederick Dainton's report on the organization of civil science within the government, for it will still be necessary to decide on what scale the government should attempt to strengthen the competitiveness of British industry by means of publicly sponsored research and development. In passing it must be said that on what seems to be the threshold of membership of the European Economic Community, it will be absurd if the British Government chooses to eschew one of the few then permissible methods of stiffening domestic industry. In short, and whatever it may think at present, the British Government is going to need not merely the efforts of its scientists but their goodwill as well.



What Future for Euratom?

ONE of the least dazzling parts of the British Government's policy declaration last week in favour of British membership of the European Economic Community is the passage dealing with what is formally known as the European Atomic Energy Community, Euratom for short. The case for a British link with the EEC is by now so self-evident that it hardly needs to be made, yet the case for membership of Euratom is so meagre as to be non-existent. Even the more enthusiastic supporters of the European venture should acknowledge that, if only so that there shall be no great disappointment over what happens—or, more likely, does not happen—in the years ahead. For the truth is that the issue of Euratom membership is so nearly an irrelevance as to be an embarrassment. It would be in everybody's interest if the prospect of four extra members were now taken as a sign that the time has come to put Euratom on a sounder footing than in the past ten years.

The greatest disappointment has, of course, been the failure of Euratom, even when supported by the full political weight of the European Commission, to arrange for seemly collaboration in nuclear power development on a European basis. To begin with, in the late fifties, there seemed every chance that an organization such as Euratom would have useful work to do. Pre-Gaullist governments of France, urged on by a liberally minded Commissariat de l'Energie Atomique, were all for international collaboration, while the then government of Germany regarded Euratom as a means of making up for time lost during the long postwar uncertainties. In the late fifties, the pace of industrial development in Italy had begun to quicken in fields such as petrochemicals and plastics, so that there too the chance of moving ahead quickly in nuclear energy seemed entirely welcome. In the event, French hankering after nuclear independence in the military field proved to be a serious stumbling block. So too, by the mid-sixties, had become the sneaking suspicion or at least greed in the heart of every European government that there were rich pickings to be had in the design, construction and sale of nuclear reactors. In retrospect, it is just possible that these centrifugal tendencies could have been beaten off by a sufficiently imaginative and forceful organization in Brussels, but it is now even more clear than it appeared to be at the time that the first plans laid by the Euratom organization were too ambitious and too hastily prepared. Schemes like that for developing a reactor cooled by organic liquids were imaginative, it is true, but it would have been more prudent if in its early days Euratom had cut its teeth on projects more likely to be successful. More modest plans would also have been more winsome, especially for those national organizations threatened by the then prospect that Euratom might succeed in all it tried to do. But in the event, things have turned out quite differently. The research programme has for the most part failed.

To be sure, there are some redeeming features, the chief of which is the cooperative programme for research on thermonuclear fusion. Evidently it is easier for nations to collaborate with each other when their national interests appear to be less directly threatened. The investment of Euratom countries in the Dragon reactor will probably

be, in the long run, a benefit, hard though it may turn out to be to quantify it. The most serious complaint against the way in which money has been spent on collaborative research in the past fifteen years is that Euratom has conspicuously failed to prevent the separate development of fast reactor projects in France, Italy, Germany and—now—the United Kingdom. As a monument to that failure, however, there remains the large research centre at Ispra (in Italy) with a staff of close on 2,000 and with no clear continuing reason for existence. In the circumstances, it is no wonder that the European Economic Community is making heavy weather of the task of working out a forward plan for Euratom. Each year since 1969, there has been talk of a long-term budget within which Euratom and its research stations could settle down to a useful job, and as a part of that process, governments, officials and industries have made what seems to be a sport of trying to suggest what kinds of work Euratom should now undertake. Some would make Ispra into a centre for environmental research. Others would turn it into a kind of international standards laboratory. But somehow the five year budget never materializes. If Mr Harold Wilson and those who oppose British membership of the EEC will allow the concept of British membership to become real, there is every chance that the negotiations now under way in Brussels within the EEC will lead to a three-year plan to tide over the period between now and the point at which Britain would be a full member of Euratom at the end of 1973.

What needs to be accomplished? The most valuable and certainly the most durable of the functions of Euratom is the safeguards system by means of which the community attempts to keep track of nuclear materials within the member countries. It is true that the safeguards system has never been quite the thing it might have been since the Government of France declared that some method must be found of excluding military explosives from the safeguards provisions of the Euratom treaty. Since then, it has never been quite clear to what extent Euratom is allowed access to French production plants on the understanding that the information gathered should not be publicized. Another possibility is that there are substantial parts of the French military explosives programme which are never included in the safeguards net. Moreover, it is not clear at present how much the Euratom safeguards will be transformed by the negotiations now under way with the International Atomic Energy Agency with a view to substituting Euratom controls for those that will in due course be administered from Vienna. The result is that nobody can safely predict how British membership of the EEC would affect the operations of British nuclear establishments. Would, for example, the fusion plants at Capenhurst now making enriched uranium for civil purposes be subjected to international inspection even though Britain is formally exempted from the provisions of the Non-Proliferation Treaty on the grounds of being or having been a nuclear power? These and many other questions like them are at present exceedingly obscure. The Euratom safeguards system, by all accounts the best feature of the community's activities, is plainly in need of making clearer and simpler if it is indefinitely to survive.

No Jobs for the Boys

A GREAT many active researchers in the United States are still able to remember and even to recall with a mixture of bravado and nostalgia how they were first compelled to find jobs in unlikely and sometimes unsuitable occupations—schoolteaching, salesmanship or shopkeeping. In part, these hard luck stories are an illustration of how deeply the depression of the thirties left its mark on all kinds of professional people then beginning their careers; when jobs are scarce, the newcomers inevitably find themselves at the back of the queue, with the result that whole cohorts of trained people may occasionally find themselves doing jobs for which they were not trained and which sometimes require no skill at all. But the way in which the tales of old heroism are remarked on is also a sign of the professionalization of science in the past few decades. It has now become less easy for men and women trained as scientists to earn their livings in other jobs. For one thing, training lasts so much longer that it counts as a valuable investment of time if not of money to new entrants to the labour market. Second, there is a real sense in which an interrupted career in professional science may be irreparably damaged.

The figures for unemployment in the United States now published by the National Science Foundation (see page 150) fit neatly into the pattern of employment prospects to be expected at times of economic recess. Among scientists as a whole, the rate of unemployment has increased—almost doubled—in a year but is still much better than that for the labour force as a whole (6.5 per cent in the first quarter of 1971). Skill is evidently an advantage still, which is borne out by the way in which people with PhD degrees had one of the lowest unemployment rates of all—1.4 per cent in the first quarter of the year compared with 3.5 per cent for less highly qualified scientists. Evidently one moral to be learned from this is that a person who has embarked on a career as a scientist will not benefit his employment prospects by refraining from completing a PhD degree.

True to the pattern of recession, the statistics also show that newly qualified people are the hardest hit, and there is nothing in the statistics to show that American employers have accepted the demands of the Women's Liberation. (In the beginning of 1971, the unemployment rate was twice as high among women scientists as among men scientists.) Predictably, many of the unemployed had been shaken out of defence and aerospace. The sobering tendency in the statistics now published is that which shows the great duration of the period of unemployment—an average of seven months by the beginning of June this year. With the next crop of graduates about to tread on the heels of those still left over from 1970, it is plain that the present recession must leave a lasting scar.

What solution can there be? No amount of vigorous action, such as that intended by the host of bills now bemusing Congress with proposals for spending money on the retraining of scientists and engineers in the gentle causes of the fight against pollution, will make up for the interruption of young people's careers and the destruction of their expectations that the present recession has caused. Unhappily, however, it helps very little to do what the Administration has done and to argue that, if there are not enough jobs to go round, the best solution is to

restrict substantially the numbers of stipends available for those wishing to enter graduate schools. On the face of things, the incidence of unemployment is greatest among the age groups from 25 to 35. Only some of these, at most a third or something like 1,000, will be people with PhD degrees who have left graduate schools in the sixties. Moreover, most of these are people trained in chemistry or physics—the incidence of unemployment in other fields is much more modest. Alarming though the figures may be for the individuals concerned, they are extremely small compared with the annual production of PhDs in the United States in recent years—18,000-odd in all the sciences and engineering in the academic year now finishing. But in any case, as the figures themselves show, a PhD degree is an advantage, not a handicap, in looking for a job, at least in comparison with a lesser qualification. The case for reducing the level of support for graduate schools is, in short, entirely independent of the present employment.

100 Years Ago



LETTERS TO THE EDITOR

[The Editor does not hold himself responsible for opinions expressed by his Correspondents. No notice is taken of anonymous communications.]

Cotteau's "Echinides de la Sarthe"

A NOTICE of Cotteau et Triger's *Echinides de la Sarthe* in a recent number of NATURE (June 15, p. 120) is likely to convey a false impression of the accuracy of M. Cotteau, and throws considerable doubt on the value of his work. It is not often that French scientific men are as conscientious as he is in the examination of authentic types. There is hardly a collection of fossil Echini which M. Cotteau has not examined; and his thorough acquaintance with all that has been written on his subject, as well as his intimate correspondence with the principal echinologists, is a sufficient guarantee that no important memoir (such as Wright's monograph) could have escaped him. Anyone who will take the trouble of turning to Cotteau's work (p. 111) will find, under *Pseudodiadema hemisphaericum*, a notice of Dr. Wright's figure of the same species (so much superior, with many others, to Cotteau's?) and a reference to his description. Nor is this an isolated case. Throughout the work M. Cotteau discusses and criticises more or less the results of this very monograph, said to have been overlooked by him. The mistake Cotteau is accused of making of assigning to Desor instead of Agassiz the specific name of *Pseudodiadema hemisphaericum* is entirely unfounded. Referring again to p. 111, we find, as a synonym, *Diadema hemisphaericum* Agass. M. Cotteau, like many continental and American writers, does not interpret the notation of species as is required by the laws of the British Association, but for that reason he should not be accused of committing mistakes which his own writings show him not to have committed. M. Cotteau, in common with others, looks upon nomenclature simply as a matter of registration; and when M. Desor transfers to *Pseudodiadema* the *Diadema hemisphaericum* Agass., M. Cotteau writes, therefore, *Pseudodiadema hemisphaericum* Desor, and not Agassiz; he may be wrong, according to the principles of the writer in NATURE, but he has not, either in this instance or in the other cases alluded to, committed a mistake through ignorance of the subject.

A. AGASSIZ

From Nature, 4, 220, July 20, 1871.

OLD WORLD

SCIENTISTS' PAY

Continuing Dispute

THE date for arbitration of the pay dispute between the members of the scientific civil service, represented by the Institution of Professional Civil Servants, and the Civil Service Department has been fixed for August 12, and, if necessary, August 13, at Church House, Westminster. The proceedings of the arbitration inquiry will not be held in public, even though the Institution of Professional Civil Servants had been hoping to arrange either that the inquiry would take place in a hall sufficiently large to accommodate some hundreds of its members, or, alternatively, that the proceedings would be relayed to a room elsewhere.

Between now and the arbitration date, the institution has also arranged a series of public meetings which are expected to provide good business for the shrunken telegraph service of the British Post Office Corporation in the shape of messages to members of parliament. With an eye to the staff of the Atomic Energy Research Establishment at Harwell, a meeting has been arranged for Friday, July 16, at the Fitzharris School, North Court Road, Abingdon (8.15 p.m.). It is hoped to arrange a meeting at Portsmouth on Monday, July 19, at the Civil Service Club and a meeting at Reading on Wednesday, July 21, at the Great Western Hotel (6.30 for 7.00 p.m.) and at the Cooperative Hall, Western Road, Weymouth, on Thursday, July 22 (7.00 for 7.30 p.m.).

The Institution of Professional Civil Servants intends to bring its campaign of publicity to a climax with a public meeting at the Central Hall, Westminster, on Tuesday, August 10.

The statistics available to the Civil Service Department do at least give the lie to the suspicion that the pay dispute has arisen at a time when the strength of the scientific civil service is declining. Since 1965-66, for example, the number of Principal Scientific Officers has increased from 1,530 to an estimated 1,748 in the current financial year. Similarly, the number of Senior Scientific Officers has increased by almost 20 per cent to a total of 1,244 and there is an even larger proportionate increase in the number of Senior Principal Scientific Officers (whose salaries are determined by a different procedure from that which has occasioned the present dispute) from 541 in 1965-66 to 668 in the current financial year. During the same period, the numbers of Senior Experimental Officers have increased even more dramatically, from 1,471 to 2,090. By comparison, the numbers of scientists

in the recruitment grades have increased less quickly over the years, suggesting that the managers of the civil service have done what they can to lessen the pressure on pay by increasing the numbers of comparatively senior posts available.

MINERAL RESOURCES

Metals or Mountains?

THE announcement last week by Sir John Eden, Minister for Industry, of grants totalling up to £50 million to aid mineral exploration of non-ferrous metal ores, fluorspar and potash in Great Britain is certain to be welcomed by the mining companies and equally certain to be regarded with reserve by the conservation lobby. The minister, in announcing the scheme, in which the government will contribute up to 35 per cent of the cost of exploration and evaluation, stressed that the normal planning controls under the Town and Country Planning Acts would still apply. In spite of these reassurances, such a scheme, with full ministerial backing, is bound to increase the pressure to grant planning approval in any part of the country.

The confrontation with the conservationists arises because these minerals are found in older rocks which make up the mountains of Britain. These are also the areas where most of the National Parks are found. Before the present scheme was announced, the Rio Tinto-Zinc Corporation applied for permission to prospect within the Snowdonia National Park. The inquiry into their application was held last March and the decision of the Secretary of State for Wales is expected soon.

The announcement of the scheme comes at a time when the richer and more easily mined ore fields in other countries are being worked out. There has been no large scale mining in the UK since the end of the nineteenth century when the low quality of ore coupled with the mining expansion abroad eventually made it uneconomic to continue mining. But developments since then in mining techniques as well as the high cost of importing these minerals (£600 million a year) have made it economic to mine low quality ores.

Some lead and zinc are being produced as by-products of fluorspar mining in Derbyshire but the only non-ferrous metal currently being mined on any scale in Britain is tin in the west of England. There are, however, known to be deposits of copper, tungsten, gold, barium and uranium. The extent and quality of the known deposits are mostly unknown and the aim of the grants is to encourage mining

companies to prove the mineral resources of Britain.

The government will recover the grant with accumulated interest if a successful mine results from the exploration. Sir John also pointed out that as well as the economic advantages of new mines, chiefly on the balance of payments, there would also be more jobs in parts of the country where they are most needed. Such advantages must, however, be weighed against the possible damage to the countryside if wholesale mining were allowed to take place.

Stars in Rock

by our Soviet Correspondent

ANCIENT rock carvings in Armenia (dating from 8000-1000 BC are reported to have revealed a considerable degree of astronomical knowledge in their makers. The carvings, which have been found at various sites in the Armenian highlands, indicate that the then inhabitants of the region divided the year into twelve parts, revealing a fairly good understanding of the length of the year and of the lunar month.

The most detailed astronomical carving is one in the Martuni region of the Sevkar foothills. Here the carving is exceptionally deep and a great deal of detail is preserved. It shows, on the left, the three bright stars of *Cygnus* in a line, with the sizes of the stars roughly proportional to their apparent brightness. Beside them is a pictogram of two men holding a serpent above their heads. This, says Dr B. Tumanyan, Director of the Observatory of the State University of Erevan, indicates the great antiquity of the constellation name of the Serpent-Bearer (*Ophiucus*).

In the top left hand corner of the picture, there are numerous dots and crosses representing the stars of *Delphinus* and the part of the Milky Way between *Aquila*, *Delphinus* and *Sagitta*. At the top there are pictograms of a sword, a shield and two crosses and a triangle, in the section where *Cygnus*, *Lyra* and *Vulpecula* should lie. The layout makes it quite clear that the pictograms represent the stars of this area, and thus indicate very primitive groupings and names for these stars.

According to Dr Tumanyan, detailed study of the carving suggests that it dates from c.3000 BC.

NEW WORLD

Cancer Compromise Almost Unites Senate

by our Washington Correspondent

THE tussle between Senator Edward Kennedy's Health subcommittee and the Administration over the organization of cancer research ended last week in an atmosphere of mutual backslapping. A compromise bill in the name of the Administration went through the Senate with only one lonely voice raised in dissent, and if the bill survives the rest of the Congressional mill in its present form, hundreds of millions of dollars a year will be poured into an independently funded cancer agency based on the present National Cancer Institute. The compromise, worked out behind the scenes of the Kennedy Committee (see *Nature*, 231, 414; 1971), seeks to set up an agency unfortunately and misleadingly called the Conquest of Cancer Agency, geographically located within the National Institutes of Health, but with a separate budget and with a director who is responsible directly to the President. A moot point, and the one on which Senator Gaylord Nelson of Wisconsin based his opposition to the bill, is whether the agency will in practice operate within the NIH, or whether the administrative arrangements that have been worked out for it will take it essentially outside the confines of the other nine institutes which together make up the NIH.

But one of the more disturbing aspects of last week's debate—if a seventy-nine to one split can be termed a debate—is that the Conquest of Cancer Agency has been sold to the Senate and the public as a measure which has a good chance of leading in the near future to a cure for cancer. The suggestion is that a massive infusion of capital into a NASA-style agency charged with the task of finding a cure for cancer is all that is needed to come up with an answer to the cancer problem. That was certainly the impression unashamedly put out by Senator Jacob Javits of New York, a member of the Kennedy committee and a co-sponsor of the bill, when he told his fellow Senators that "Senators Dominick, Kennedy and I, and a majority on the committee, feel that we are so close to breakthroughs in cancer, that cancer represents to the people such an awesome disease or illness, that we must, as a national effort, quite apart from the scientific question, undertake a crash programme under a unified direction, to make the final

breakthroughs. . . . The country finds the death rate from cancer unacceptable because it thinks it is imminently avoidable. That is the question we think we are close enough to breakthroughs on, and that one final drive will do it."

There is a real fear in the biomedical community that if the cancer cure agency is sold to the public in these terms, and fails to come up immediately with the answers, scientists and science alike will be discredited. The fact that a so-called cure for cancer is probably not as attainable as the Moon could come as a rude shock to those who will be looking for the proposed agency to produce the right answers at the mere wave of the financial wand. These sentiments were eloquently expressed only last month by Albert Sabin, founder of the oral polio vaccine, who has been widely tipped to head the new cancer agency. Speaking at a meeting in Brussels, Sabin said that researchers working on such targeted research are worried because they have been charged with a task which to the public seems to be a question merely of finance, but which in practice is much more complicated.

Those sentiments apart, the passage through the Senate last week of the Administration's bill is an object lesson in Congressional politics. The tussle between President Nixon and Democratic Congressmen led by Kennedy has already been well documented in *Nature*, but which side has in practice won the battle will probably not become clear until the proposed agency has been working for some time. At the root of the disagreement is whether or not the Conquest of Cancer Agency will remain in practice as well as in theory a part of the National Institutes of Health. Kennedy originally sponsored a bill in the Senate (numbered S34) which proposed in essence that the National Cancer Institute should be taken outside the National Institutes of Health and raised to the status of an independently-funded agency reporting directly to the President. But the Administration later introduced a bill (numbered S1828) which proposed instead that the National Cancer Institute should remain as a bureau within the NIH, and that it should have a separate budget. The bill which was passed by the Senate last week was something of a compromise between

these two positions, although it retained the number S1828, meaning that it was the Administration's bill.

Kennedy's proposals met with the almost unanimous disapproval of the scientific community, whose witnesses before the Health subcommittee deplored the suggestion that the proposed cancer agency should be taken out of the NIH and away from other biomedical research. And it is on this point that Kennedy has been seen to come into line with the Administration's thinking, for the bill which went through the Senate called for the setting up of the Conquest of Cancer Agency within the NIH, but with a director reporting to the President. The bill in particular lays down some specific guidelines for cooperation between the Conquest of Cancer Agency and the NIH. The most important is that the director of the agency is authorized to "take necessary action together with the Director of the National Institutes of Health so that all channels for the dissemination and cross fertilization of scientific knowledge and information existing prior to the effective date of this Act between the National Cancer Institute and the other Institutes of Health shall be maintained between the Agency and the Institutes of Health to ensure free communications between cancer and other scientific, medical and biomedical disciplines".

But these provisions do not satisfy Senator Nelson, who argued and voted against the bill, and Senator Cranston from California, who expressed doubts but voted for the bill. Nelson made it clear that he believes that it is the Administration which has sold out to the original Kennedy proposal rather than the reverse. Referring throughout his remarks to the proposed agency as an institution separate from the NIH, Nelson warned that other institutes within NIH will want similar treatment, as in fact the National Heart and Lung Institute has already requested, and this bill, if it emerges unchanged by the House, will signal the break-up of the NIH itself. "I have repeatedly asked the proponents of this measure—that is, the proponents of creating an independent institute outside of NIH—just what is to be gained by it . . . there is not much of a reason for it except a kind of emotional commitment to do something with an independent institute that they think cannot be done if they

remain within the NIH, the finest institution of its kind on Earth," Nelson said.

Another important plank in Nelson's platform is that under the terms of the bill, the Director of the NIH will be responsible for about \$100 million worth of cancer-related research within the nine remaining institutes of health, while a further \$332 million will be spent by the proposed Conquest of Cancer Agency housed within the NIH but reporting directly to the President. "It does not make much sense at all," he said. "I think that if everybody sat down and thought about it for a while, they would recognize that this is not the best approach to accomplish the end we all seek." Cranston echoed these sentiments, believing that the suggestion for cooperation between the director of the proposed agency and the Director of the NIH is insufficient. "What is needed is statutory authority making clear that the Director of NIH should have the formal responsibility of submitting the cancer programme and budget to the President, along with his evaluation of the cancer programme and appropriate recommendations," he said.

In the event, however, the bill went through with only Nelson recording his dissent, and the nature of the compromise worked out between the supporters of Kennedy's original bill and those who acceded to the Administration's original proposals can be evinced from an extraordinary tale told by Javits. "Many people always think that we are very strong minded and that we want to have our *imprimatur* on everything and will never yield a single prerogative or permit the name of anyone else to get on a bill except our own names, but in this instance we had finally to prevail on the Senator from Colorado (Dominick), who felt he wanted the Senator from Massachusetts (Kennedy) to bring the bill to the floor."

UNEMPLOYMENT

Grim Prospects

by our Washington Correspondent

THIS year's crop of science and engineering graduates who have already found themselves swelling the ranks of the unemployed do not need to be reminded that they have been thrown on to the job market at its most depressed time since the Second World War. The full extent of unemployment at present remains, however, a subject for speculation, but some alarming indications can be gleaned from a survey conducted in May by the National Science Foundation, the results of which have now been made public.

Table 1 Number and Rates of Unemployed Scientists, by Field of Science, 1971

Field of science	Responding	Not employed and not seeking employment *	In labour force	Unemployed	Unemployed rate (%)
Total	253,078	8,003	245,075	6,337	2.6
Chemistry	73,008	2,576	70,432	2,101	3.0
Earth and marine science	18,724	535	18,189	486	2.7
Atmospheric and space science	5,243	198	5,045	142	2.8
Physics	30,220	1,089	29,131	1,126	3.9
Mathematics	19,745	642	19,103	491	2.6
Computer sciences	8,840	223	8,617	309	3.6
Agricultural sciences	12,708	247	12,461	110	0.9
Biological sciences	39,871	855	39,016	656	1.7
Psychology	20,319	731	19,588	304	1.6
Statistics	2,465	92	2,373	51	2.2
Economics	9,767	318	9,449	154	1.6
Sociology	5,512	251	5,261	196	3.8
Political science	4,286	134	4,152	141	3.4
Anthropology	1,029	48	981	13	1.3
Linguistics	1,341	64	1,277	57	4.5

* Includes such groups as retired, students, housewives, and others not in labour force.
Source: National Science Foundation, National Register of Scientific and Technical Personnel.

Based on returns to a questionnaire sent out to 300,000 scientists, the NSF's conclusion is that 2.6 per cent of all qualified scientists were out of work in the spring of 1971. Compared with a national average unemployment rate of 6.5 per cent during that period, these findings may not evoke too much sympathy on the dole queues. But the important point is that they correspond to the period immediately before this year's new graduates came off the university production line, and with the abundant reports of cutbacks by industry in graduate recruitment, they are cold comfort for new graduates seeking employment. The estimates should also be considered as minima, for they are based on replies from scientists who could be reached, and they do not take account of the fact that many scientists were underemployed or had left science for other sectors of employment.

Chief among the findings are:

- 1.4 per cent of all doctorates were out of work this spring, compared with 0.9 per cent last year, while the unemployment rate among nondoctorate scientists was 3.5 per cent compared with 2.9 per cent in 1970.

- Young scientists were the hardest hit, with an unemployment rate of 5.3 per cent.

- The unemployment rate among women was running at 5.2 per cent, compared with 2.3 per cent for men.

- About 45 per cent of unemployed scientists said that their last science-related employment was supported to some extent from government funds.

- Scientists who are not citizens of the United States reported an unemployment rate of 4.2 per cent, compared with 2.5 per cent for US citizens.

Chemists and physicists between them accounted for half the total of

unemployed scientists in the survey, and unemployment rates in these two sectors were running at 3.0 and 3.9 per cent respectively, compared with 1.5 and 2.3 per cent in 1970. Sociologists, political scientists and computer scientists also reported unemployment rates greater than 3 per cent (see Table 1). It also seems that unemployed scientists who were previously employed by industry have been most affected by the present job shortages, for in 1970 industry and business employed 31 per cent of the scientists in the national register, but 38 per cent of the unemployed scientists in the NSF survey gave industry and business as their previous employers.

The hardest hit area is California, where 42 per cent of the unemployed scientists were located, and in Seattle, Washington, the unemployment rate among scientists was running at 5.6 per cent in the spring. The Department of Labor has in fact designated 14 areas as being severely affected by unemployment of scientists, engineers and technicians, and the chief factor linking many of the areas is a high concentration of defence and aerospace activities. The areas include Orange County, California, Seattle, Dallas, Long Beach, California, Atlanta and Cape Kennedy.

Short Notes

THE Kitt Peak National Observatory has a new director in the person of Dr Leo Goldberg, director of the Harvard College Observatory. Dr Goldberg will succeed Dr Nicholas U. Mayall on September 1. Dr Goldberg, who is an astrophysicist specializing in atmospheric problems, has divided his career between Harvard and the University of Michigan.

NEWS AND VIEWS

Circular Polarization and Astronomy

THE possibility that circular polarization measurements can provide significant new information about the planets has now been taken a step further. On page 165 of this issue of *Nature*, Kemp and his colleagues, who have been working at the Mauna Kea Observatory, Hawaii, report further measurements of the circular polarization in the light reflected from Jupiter, and the first measurements of circularly polarized light from Mars, Venus, Mercury and the Moon. To begin with, their new study verifies the explanation of the circular polarization from Jupiter that was put forward tentatively when the discovery was announced two months ago (*Nature*, **231**, 169 and 170; 1971). It also seems probable that the circular polarization recorded from the other planets can be explained by the Jupiter mechanism, or a modification of it. But with positive measurements from several planets now under their belts, Kemp and his colleagues allow themselves a little more speculation about the applications of their discoveries to studies of the planets than they did two months ago.

In the case of Mars and the Moon, the occurrence of circular polarization in the scattered light appears to be attributable to their dust-covered surfaces, so that the measurements are unlikely to provide any information that is not already known. The same explanation probably holds for the single observation of circular polarization from Mercury that Kemp and colleagues report, although in this case any information gathered is likely to be more valuable. It remains to be seen whether the well known awkwardnesses that arise in observing a planet so near the Sun will prove to be serious obstacles.

The data from Jupiter and Venus, and the possibility of obtaining data from Uranus and Neptune, are likely to be more important, however. In the case of Jupiter and Venus, the origin of the circular polarization has to do with their dense atmospheres (as is also the case for measurements of Mars at blue wavelengths where the effect of the thin Martian atmosphere seems to become significant). The mechanism that Kemp and colleagues put forward two months ago for the origin of the circular polarization from Jupiter has now been essentially verified by the way in which the circular polarization from the northern and southern hemispheres of the planet altered through the period of opposition (opposition was on May 23). The way is now open, then, for a detailed examination of the variation in circular polarization across the surface of Jupiter when the equipment for examining small areas of planet is available. Already, however, there is interest in the possibility of significant variation in the magnitude of the circular polarization from the vicinity of the Red Spot. The Red Spot is in the southern hemisphere of Jupiter, and Kemp and colleagues report unusual behaviour of the circular polarization averaged over the entire southern hemisphere with which the Red Spot may conceivably be related. Kemp and colleagues have already reported evidence that the leading edge of the Red Spot is more strongly circularly polarized than the trailing edge (*Nature*, **231**, 169; 1971).

The outlook then is promising that circular polarization measurements, particularly of the outer planets, will

provide work for some time to come. Historically this prospect is an extension of research that has been carried out by Kemp and others for the past year or two on circular polarization in the light from white dwarf stars. On the face of it, the detection of circular polarization from white dwarfs is at present likely to be of more significance than the planetary work, largely because the virtually featureless spectra of many white dwarfs are a bar to a better understanding of their nature. Through the work of Kemp and others, however (see *Nature Physical Science*, **231**, 21; 1971), it is now clear that the circular polarization that has now been observed in the light of three white dwarfs—Grw + 70°8247, G195-19, and G99-37—can be related to the strengths of their magnetic fields. The measurements indicate fields of the order of 10^6 – 10^7 Gauss. This work is important, if only because it lends support to an essential requirement of all pulsar theories—that the rapidly spinning neutron stars which are believed to be the origin of the pulsating signals have the enormous magnetic fields of the order of 10^{12} Gauss that are necessary for an understanding of their electromagnetic emission. Neutron stars are expected to have diameters of the order of 10 km, compared with 1,000 km for typical white dwarfs, so bearing in mind that the magnetic field increases as the inverse square of the radius as a star collapses (assuming the conservation of magnetic flux), there is now encouraging observational support for the view that the collapse of a main sequence star to a neutron star can lead to the required large fields of 10^{12} Gauss.

The circular polarization from white dwarf stars has its origin in the characteristics of blurred spectral lines in a strong magnetic field. To be sure, Jupiter is in many ways surprisingly close to being a white dwarf, but it was clear from the start that the mechanism which accounts for the white dwarf measurements could not explain those from the planets. For one thing, a field of at least 10^3 Gauss would be needed, rather than the 10–100 Gauss which seems to be indicated for Jupiter. So there are several different mechanisms in the air for the origin of the circular polarization, depending on the observed object, with much work still to be done on each of them. In the current *Astrophysical Journal*, for example, Shipman of the Hale Observatories examines Kemp's theory for the white dwarf Grw + 70°8247, and is able to modify it to explain better the wavelength dependence of the circular polarization measurements (167, 165; 1971). Where Kemp predicted that the circular polarization would increase linearly with wavelength, Shipman is able to reproduce, very roughly, the peak in the fractional polarization that is observed for this star at 4000 Å by a re-examination of the conditions in the star's atmosphere. The anomalously large circular polarization that has been reported in the infrared remains a problem, however. Clearly there is much work to be done, both by theoreticians, and at the telescope, to see how general is the form of the spectral dependence observed for Grw + 70°8247. Together with the interesting data that may come out of the planetary measurements, the circular polarization measurements are a rich seam which will be worked for many years to come.

NUCLEAR FUSION

Tokamak Excels

from a Correspondent

THE conference on plasma physics and controlled fusion research organized by the International Atomic Energy Agency at Madison, Wisconsin, from June 17 to 23 was the fourth in the series of meetings which are held at three-year intervals. They constitute the forum for the international exchange of information on progress towards an ultimate power-generating fusion reactor. At present the principal concern is the plasma confinement problem, and in spite of the title the only plasma physics covered was that held to be directly relevant to fusion.

Many different schemes for plasma confinement are under study and a good deal of attention is given at these international meetings to their relative progress and future promise. This latest meeting was fairly unremarkable in the sense that no outstanding new results or concepts were presented; the remarkable feature was that for two successive meetings in the series the Russian Tokamak system has maintained a lead both in performance and promise over rival schemes.

Thus Dr E. Meservey (Princeton University) presented results from the Princeton Tokamak which in broad terms confirmed those obtained earlier by Russian workers on their T-3 machine in Moscow. There are some differences and attention was naturally concentrated on these. For example, the electron temperature is strongly peaked near the axis in the American machine, whereas in the Russian one the profile is relatively flat. There are other differences which seem to be attributable to differences in interpretation of essentially the same experimental data. Thus the Russians think that the containment time for particles greatly exceeds that for the energy whereas the American view is that they are about equal.

Dr V. S. Strelkov (Kurchatov Institute, Moscow) presented results from the latest Russian Tokamak, T-4. This has a higher magnetic field (50 kG) and the stabilizing copper shell is nearer to the plasma than in T-3. The plasma current has thus been increased by almost a factor of two; the ion temperature as deduced from the neutron yield reaches 650 eV. With a current of 110 kA the electron temperature is ~ 2 keV, the plasma density is $3 \times 10^{13} \text{ cm}^{-3}$ and the containment time is 10 milliseconds. These are impressive numbers but they fall short by about one order in both temperature and density and two orders in containment time over the requirements for a fusion reactor. It should be emphasized that although many features of the Tokamak discharge are not understood there are fairly clear, if

expensive, lines along which the next experiments should proceed.

Whether some tortoise will eventually overtake the Tokamak here is an interesting question. The stellarator which is closely related to the Tokamak, but much more expensive and difficult to build, seems to be undergoing a random walk rather than progress. Dr P. Reynolds (UKAEA, Culham Laboratory) presented results from his small stellarator which agree very well with the theory of classical containment. By contrast Dr K. Miyamoto (Nagoya University) finds fluctuations and anomalous losses in his very similar machine. For the largest stellarator at present operating in the world, the Uragan machine at Kharkov, Dr K. N. Stepanov reported electron temperatures up to 400 eV, density $\sim 5 \times 10^{12} \text{ cm}^{-3}$ and containment time $\sim 300 \mu\text{s}$.

Research on high beta toroidal confinement is still in an early stage as groups previously working on linear theta pinches move into the field. Typical results on screw pinches reported from Jutphaas, Culham and Garching were electron density $\sim 10^{16} \text{ cm}^{-3}$,

plasma temperature ~ 100 eV and containment time $\sim 10 \mu\text{s}$. This configuration relies for its equilibrium on toroidal current flow in the outer low density region. The rapid decay of this current is believed to be the limiting factor and Dr P. C. T. van der Laan (Institute for Plasma Physics, Jutphaas) discussed a variety of possible explanations without reaching any definite conclusion. Dr H. A. B. Bodin (UKAEA, Culham Laboratory) presented results on the diffuse pinch configuration which were encouraging in the sense that the configuration could be set up and its stability agreed roughly with theory but discouraging in that the temperature was limited by impurity radiation to ~ 10 eV. Results from a segment of the Scyllac torus reported by Dr F. L. Ribe (Los Alamos Scientific Laboratory) showed control of the toroidal drift by the addition of $l=0$ and $l=1$ fields.

In mirror research the chief result was that reported by Dr F. H. Coensgen (Lawrence Radiation Laboratory, Livermore) who finds that plasma is lost from his 2X machine at about ten times the classical rate. This fast loss is accom-

Mixed RNAs in Transformed Cells

IN *Nature New Biology* next Wednesday, Wall and Darnell report a series of elegant DNA-RNA hybridization experiments which establish once and for all that sequences of SV40 RNA occur in large molecules covalently linked to sequences of host cell RNA in the nuclei of mouse cells transformed by SV40.

In 1968 Sambrook and his colleagues showed that, in stably transformed cells, SV40 DNA molecules are integrated into DNA molecules of the host genome. Since then, although the number of integrated SV40 genomes in transformed cells has remained a matter of dispute, numerous groups have found that SV40 RNA is present in both the nucleus and cytoplasm of transformed cells. Further, it is generally agreed that SV40 RNA occurs in the nucleus in molecules which are longer than a single complete SV40 genome and in the cytoplasm the SV40 RNAs associated with polysomes are no longer than a single SV40 genome. There is every reason to believe that these polysomal SV40 RNAs are derived by cleavage from the large nuclear RNA molecules which contain SV40 sequences, but how do these large nuclear RNAs themselves arise?

There are two obvious possibilities which have been canvassed. If several SV40 genomes are integrated in tandem in each transformed cell they might be transcribed as a single unit to yield RNA molecules longer than a single SV40 genome and containing nothing

but SV40 sequences. Alternatively, the SV40 genomes may be integrated singly in such a way that they are always transcribed together with some of the adjacent host DNA; in which case the size of these RNAs would depend on how much of the host genome is transcribed and they should contain host sequences either at the 5' or 3' or both sides of the SV40 sequences.

To decide this issue, Wall and Darnell first hybridized to SV40 DNA RNAs from the nucleus and cytoplasm of transformed and untransformed cells. They then recovered the hybridizing RNA from the hybrids, which must contain SV40 sequences, and tested to see if it would also hybridize with cellular DNA from untransformed cells. They detected nuclear RNA molecules from transformed cells which hybridized with both SV40 and cellular DNAs. Wall and Darnell conclude therefore that in transferred cells at least some SV40 DNA molecules are transcribed together with some host DNA to give large and mixed RNA molecules. And although their results do not prove the idea that these mixed molecules are precursors of the small SV40 RNAs associated with polysomes they do not contradict it. By further refining the hybridization techniques and ultimately by fingerprinting these various RNAs it should be possible to decide if they are related as precursors and products and to learn something of the mechanisms which control transcription and messenger RNA maturation in eukaryotes.

panied by evidence of instabilities with frequencies near the ion cyclotron frequency. Because a mirror reactor requires near-classical confinement this result is not encouraging; it is possible, however, that the necessary quiescence can be achieved by careful machine design and control of the particle velocity distribution.

Theoretical work presented at the conference dealt chiefly with plasma confinement and stability in the Tokamak. There is now a fair measure of agreement on the classical theory of confinement in symmetric low beta systems. Attention is now paid to the effect of residual fluctuations on such systems and contributions from Dr S. Yoshikawa (Princeton University) and Drs A. A. Galeev and R. Z. Sagdeev (Kurchatov Institute, Moscow) reached similar conclusions by rather different routes. This work emphasizes the role of the poloidal magnetic field in plasma confinement, the much stronger toroidal field performing only an auxiliary function. On the question of stability, the situation is not clear; a contribution by Drs D. Pfirsch and H. Tasso (Institute for Plasma Physics, Garching) which showed the Tokamak to be unstable against a simple mode will undoubtedly be the subject of much critical attention.

For the first time at these meetings a session was devoted to reactor systems. The broad conclusion from this session was that within the necessarily very wide margins of error, fusion as a power source might be competitive with other systems and might offer substantial environmental advantages.

RNA

Messenger's Burden

from our Molecular Biology Correspondent
ONE of the strangest molecules that explorers of the eukaryotic nucleus have brought to light is polyadenylic acid, in almost monodisperse form. This observation became more than a mere unexplained curiosity when Lim and Canellakis reported last year that haemoglobin messenger RNA contains a long tract of poly A, which apparently largely accounts for the discrepancy between its observed and expected molecular weight.

The question of how general this phenomenon may be has now been taken up in several laboratories, and a clutch of three articles on the subject is to be found in the current issue of the *Proceedings of the US National Academy of Sciences*.

Lee, Mendecki and Brawerman (68, 1331; 1971) have examined the messenger RNA of ascites cells. They find that at pH 9, though not under more nearly neutral conditions, a large proportion of the rapidly labelling

RNA fraction of polysomes is specifically trapped on a membrane filter. The fraction so obtained leaves after digestion with pancreatic or T₁ ribonuclease a sedimentable core consisting largely or wholly of adenylic acid residues, a label in adenosine being almost completely recovered in this resistant fragment, whereas a label in uridine vanishes. Moreover, the behaviour of synthetic poly A on the membrane filters makes it clear that it is the poly A segment that causes the messenger to be retained. The poly A core recovered from the messenger sediments at about the same rate as tRNA. Another property of the messenger, conferred on it by its poly A, is its tendency, depending on the salt conditions, to enter the wrong phase during phenol extraction.

Very similar results were obtained by Edmonds, Vaughan and Nakazato (*ibid.*, 1336) for the messenger RNA of HeLa cells. The poly A core was shown by polyacrylamide gel electrophoresis to have a very narrow molecular weight distribution, and an esti-

ated length of 150–200 nucleotides. This is in fact rather too high to be accounted for by the estimated poly A content of the messenger, but it does not necessarily follow that some of the messenger is devoid of poly A: a number of other explanations are possible, for example that some of the label goes into other kinds of RNA than messenger.

The chief object of the article, however, is to treat the poly A as an identifying feature, and to look for its presence in the rapidly labelling poly-disperse high molecular weight RNA present in the nucleus, which many workers in the field would like to believe is a messenger precursor. Poly A is indeed recovered from this material by digestion, and is of the same size as that found in the messenger. It makes up only some 0.5 per cent of the total. That the poly A is a part of a large RNA molecule was demonstrated by its failure to dissociate and redistribute itself between labelled and unlabelled nuclear RNA after addition of a high concentration of an organic

Acceleration of Cosmic Rays

THE drawback of the venerable Fermi mechanism for the generation of the cosmic ray spectrum is the large energies that the particles need to have before the mechanism comes into play. Although the scattering of cosmic ray particles by irregularities in the magnetic fields of interstellar space will produce an energy distribution which matches the experimental data on cosmic rays, as Fermi suggested the particles have to have initial energies of 200 MeV if they are protons, and higher energies if they are heavier particles.

A process which will raise the energies of particles from thermal energies to sufficiently high energies for injection into the Fermi mechanism is suggested in next Monday's number of *Nature Physical Science*. A group at the University of Bologna argue that particles able to take part in the Fermi mechanism may come from the nucleus of the galaxy, where they have been accelerated from thermal energies by interactions with the magnetic fields of neutron stars.

This notion seems to be inspired by the pulses recorded by Weber's gravitational wave equipment, and whatever the explanation of the data published by Weber, his results have stimulated theoretical work on the conditions in the nuclei of galaxies. Interactions among clusters of collapsed objects at the centre of the galaxy might be a source of gravitational waves, but there are other more prosaic reasons for supposing that the nuclei of galaxies contains dense matter. In any case, the

Bologna group consider a cluster of neutron stars, a proportion of which have strong magnetic fields, and show how charged particles can gain energy by being scattered by the fields.

Protons need to be scattered only twice to gain the 200 MeV that is the entry ticket for the Fermi process. Heavier particles may need to be scattered more than twice, but there is no reason why the 1 BeV, 20 BeV, and 300 BeV that are the required injection energies for alpha particles, oxygen, and iron respectively should not be achieved in this way.

Little more can be said without knowing more about the abundances of the elements in the interstellar gas in the galactic nucleus, and little light is likely to be thrown on this aspect of the problem for many years. The hope is, of course, that the abundances, together with the fact that it becomes progressively more difficult to provide the heavier elements with the necessary injection energy, will provide a match with the element abundances observed from measurements on cosmic rays.

The Bologna article is well in tune with recent trends in astrophysics. The beginnings of a realization of the parts played by high energy processes and by collapsed objects are suggesting several ways in which the acceleration of cosmic rays may be accomplished. For example, particles thrown out of the atmospheres of pulsars by their rapid rotation rates may also have high enough energies to take part in cosmic ray processes.

solvent which causes the breakdown of base pairing. When RNA synthesis is stopped by actinomycin D, the poly A is also largely suppressed. Evidently therefore the poly A is transcribed from the DNA as part of the RNA chain, and is not added afterwards.

These results then provide support for the messenger precursor hypothesis, and agree with yet another study with a similar aim by Darnell, Wall and Tushinski (*ibid.*, 1321). Working with HeLa cells they too find poly A in the messenger, and in small quantity in the nuclear RNA. They have also hybridized both species of RNA with the endogenous DNA under conditions that lead to the detection only of molecules with sequences multiply "reiterated" in the DNA. The hybridized RNA is then isolated by digesting away the unbound part with ribonuclease. The hybridized messenger is enriched in A, compared with total messenger. It seems then the poly A is a part of, or at least adjacent to, the rapidly hybridizing RNA sequence, otherwise it would be liberated during the nuclease step.

The function of the poly A tract in the messenger is a matter of conjecture. Because the messengers are monocistronic and the poly A is not translated, it is likely that it occupies one end of the RNA chain. From the length of the haemoglobin message and that of the poly A tract, it seems clear that there is no room for the elaborate "leader" sequences that are found in viral RNA messengers, and are of uncertain function. The poly A may be required for translation, or for attachment to the messenger, involving perhaps the group of proteins that appear always to be attached to the messenger, without evidently getting in the way of the translation machinery. This RNA-protein package, in fact, seems to be the form in which the messenger is expelled from the nucleus, and the possibility that the function of the poly A relates to the transport of the messenger into the cytoplasm may also be entertained.

BACTERIOPHAGES

Pathway to a Phage

from our Cell Biology Correspondent
ANYBODY who has looked at high resolution electron micrographs of bacteriophage T4 particles and marvelled at their intricate architecture can readily understand why more than one molecular biologist has been content to devote years fathoming out the genes involved and the steps in their morphogenesis. And the story they have to tell is becoming as fascinating as the T4 phage is intricate. The tally of genes required for T4 morphogenesis has now reached 46, no less, and that

is a minimal estimate. In the current issue of the *Journal of Molecular Biology* (58, 685 and 693; 1971) two groups, Meezan and Wood at CalTech and King at the MRC Laboratory at Cambridge, describe in detail the functions of just four (genes 48, 54, 19 and 3) of the twenty-one genes involved in the assembly of the phage tail.

Both groups find that in *Escherichia coli* infected with strains of T4 carrying amber mutations in any one of genes 48, 54 or 19 phage baseplates—hexagonal structures with six projecting spikes which in the mature phages are attached to the tail core and its surrounding sheath—accumulate, but complete tails do not form. Because extracts of cells infected with one or other of these mutants complement and yield some infectious phage the proteins specified by these genes are able to catalyse tail formation *in vitro*. Moreover such complementation experiments indicate that the products of these three genes are required in a unique order (gene 48 product first, followed by gene 54 product and finally gene 19 product) for the polymerization of the tail core on the baseplate. Exploiting temperature sensitive mutants, King has also shown that gene 19 specifies the major structural protein of the tail core and the proteins specified by genes 48 and 54, not themselves part of the core, somehow act on the baseplates rendering them a suitable substrate for the polymerization of the gene 19 structural protein.

King has also determined the role of another gene, 3; its product is not involved in the polymerization of the tail

core, neither is it the structural protein of the surrounding tail sheath (that is specified by gene 18) but the gene 3 product does react with the terminal annuli of the tail core in the absence of the sheath. It seems that the gene 3 product acts on the free end of the core and converts it to a substrate for the attachment and stabilization of the sheath protein. And once these four genes have acted in the correct order and the baseplate, core and sheath have assembled, the product of yet another gene, 15, can form the actual joint linking the sheath to the tip of the core.

As King takes pains to point out, the assembly of the tail of T4 depends on a sequence of interactions between particular gene products and a macromolecular precursor structure; apparently the various structural proteins, unable to interact strongly when in solution, strongly interact with the macromolecular substrate produced by the preceding step in the morphogenetic pathway. The gene 15 protein, for example, which joins the terminal subunits of the tail core and sheath does not react in solution with the proteins specified by genes 18, 3 and 19, but strongly interacts with them once they are assembled into a sheath and core attached to the baseplate. Presumably during assembly these proteins undergo either allosteric or chemical changes which make them suitable substrates for, and able to interact with, the next protein to be polymerized. Such a highly regulated pathway, which may well be the evolutionary consequence of the fact that the phage is assembled at sites distant from those where the

Sequence and Synthesis of Substance P

It has been known for forty years that a substance, identified only recently as a peptide, and present in a variety of tissues, possesses hypotensive and a variety of other physiological activities. Appreciable amounts of this material are hard to come by, but in view of its broad neural activity, which is quite different from those of the hypothalamic peptide hormones, the determination of its structure is of considerable interest. The complete sequence is reported by Chang, Leeman and Niall in next Wednesday's *Nature New Biology*. The sequence is arg-pro-lys-pro-gln-gln-phe-phe-gly-leu - met - NH₂. Preparations derived from three different bovine tissues were found to have the same sequence. Substance P is inferred to occur throughout the central nervous system.

The sequence is also compared with those of two other peptides of similar activity, one, physalaemin, from the skin of an amphibian, the others eledoisin, from the salivary glands of

a cephalopod. These also have eleven residues, of which four, including the last three at the N-terminus, are identical in all three molecules. In most other positions there are conservative substitutions. Physalaemin and eledoisin have pyrrolidone carboxylic acid as the C-terminal residue.

In a second article Tregear *et al.* describe the synthesis of substance P by the solid-phase Merrifield method. The product is active in respect of the various functions associated with natural substance P. This work provides a good example of the value of modern peptide synthesis technique, for it makes available in relatively large quantity a biological material which can otherwise only be isolated in very small amounts. The authors point out the possibilities now open for its biological characterization. The synthetic material will be used to prepare antibodies, with the aid of which it should be possible to locate and assay it in nervous tissue and elsewhere.

structural proteins are synthesized, has obvious selective advantages. Phage heads, for example, cannot attach to tails unless they are complete; heads cannot therefore be wasted in incomplete and uninfected particles. Likewise sheath protein cannot polymerize on baseplates until the baseplates have initiated the polymerization of a tail core. With every successive step rigorously dependent on all its predecessors the phage assembly line is optimized for the production of complete and infectious progeny.

CONTINENTAL DRIFT

Ice and Gondwanaland

from our Geomagnetism Correspondent

GOUGH'S recent novel suggestion (*J. Geophys. Res.*, **75**, 4475; 1970) that the Carboniferous ice cap may have been the agent which initiated the break-up of Gondwanaland is now severely criticized by Meyerhoff and Teichert (*J. Geophys. Res.*, **76**, 4038; 1971), who rather unexpectedly take the view not that the ice cap was incapable of doing any such thing but that the idea of a large continuous Carboniferous ice sheet is not valid in the first place.

The gist of Gough's original contention was that the stresses produced by a large ice sheet could have fractured the lithosphere and initiated separation of the fragments and could then have caused the flow in the asthenosphere which allowed continental drift to continue. At the same time, the asthenospheric material would have risen in the crustal fractures to form the embryonic ocean floors. To support this idea Gough performed a simple first-order calculation for the stresses produced by an ice sheet 2 km thick having a diameter of 3,000 km and so showed that the stress differences at the surface under the edges of such a load are up to 100 bars, that the largest stress difference under the centre of the load is about 130 bars and occurs at a depth of about two-thirds of the load diameter, and that at the depth of the asthenosphere the stress would be in the range 60–90 bars, far greater than the 10 bars sufficient to produce creep in the mantle.

One of the earlier and most influential authorities for the existence of a large Carboniferous ice sheet was du Toit (*Our Wandering Continents*, Oliver and Boyd, 1937) whose detailed reconstruction showed a slightly curved ice cap 11,000 km long and 4,500 km wide covering a quarter of South America, slightly less than half of Africa, most of Australia and the whole of Madagascar, India and Antarctica. Such a reconstruction has since been accepted by most palaeomagnetists. But

Meyerhoff and Teichert no longer believe it. It is their unequivocal view that "at no time during the Carboniferous and Permian did a large ice sheet occupy any part of the southern continents—with the single possible exception of Antarctica". Instead they point out that there is evidence for about thirty separate, unconnected Carboniferous and Permian ice "centres" in the southern hemisphere of which the largest, in Brazil and southern Africa, had maximum diameters of 1,600 km. Furthermore, there were apparently six in India and Pakistan and four in the Urals and north-central Siberia. The evidence for the lack of connexion between these ice centres is, according to

Meyerhoff and Teichert, that all but three were mountain ice caps with radial U-shaped valleys and that even the remaining three were surrounded by highlands. Moreover, they quote numerous references which suggest that many of the known Carboniferous and Permian ice caps were not precisely contemporaneous. In short, "the concept of a single, vast, continental ice sheet over a large part of Gondwanaland has been thoroughly disproved".

The second class of objection put forward by Meyerhoff and Teichert raises issues of a rather different kind. Thus when their isolated ice centres are plotted on a reconstruction of Gondwanaland they find that many lie in the

Role of Cytotoxic Cells *in vivo*

ONE of the more interesting developments in cellular immunology in recent years has been the recognition of several sorts of cytotoxic cells. In almost all instances the aggressive activities of such cells have been demonstrated *in vitro*; direct observation has shown that certain kinds of killer cells will seek out and eventually destroy the hapless target cells. Work on these cell populations is now advancing towards a definition of the mechanism by which cell death is brought about and towards delineation of the role of cytotoxic cells in situations *in vivo*. Three articles in the forthcoming issue of *Nature New Biology* relate to this subject.

Alexander and Evans describe how they have brought macrophage populations in contact with either endotoxin or twin stranded RNA. Subsequently, the macrophages proved capable of killing a lymphoma cell population. This intriguing phenomenon is apparently non-specific, but it does require fairly long contact between the lymphoma cells and the endotoxin or ds RNA treated macrophages. The results cannot be explained, according to the authors, by any direct cytotoxic effect of endotoxin or ds RNA on the lymphoma cells, but it could be that a cytotoxin is released from the activated macrophages.

Peter and Dawkins quite specifically invoke a cytotoxin to explain their results. Human adenoidal cells were placed in culture with PHA and the resulting supernatants were found to be cytotoxic for mouse L cells in a manner which was superficially similar to that of a known cytotoxic antibody. The cell array present in these cultures is, of course, less well defined than that of Alexander and Evans, in which an adherent macrophage population can be specifically involved as the significant cell. Nevertheless, Peter and Dawkins conclude that because the

production of their cytotoxic effect is dependent on the presence of PHA, which is noted for its capacity to transform lymphocytes, their cytotoxin derives from a lymphocyte population. It is intriguing to note that the type of lymphoid tissue placed in culture was derived from a part of the body which is subject to continuous contact with bacteria and presumably with their endotoxins. It would be interesting to learn whether lymph node cells from "quieter" parts of the body were similarly effective.

The third article, by Harding *et al.*, deals with a more specific phenomenon. Allogeneic (same species, different strain) target cells can be destroyed in culture by immune thymus-derived (T) lymphocytes (Cerottini *et al.*, *Nature*, **228**, 1308; 1970), by cells which produce cytotoxic antibody but which are not directly cytotoxic or by cells which can only attack antibody coated cells (the antibody itself is not lytic in the absence of complement). Harding *et al.* are concerned with the origin of this last kind of cell. They conclude that it is not a cell of thymic derivation because it is still present in normal numbers in animals with much reduced numbers of T cells. These authors also say that their cytotoxic cell presumably attacks not the antigens on the target cell but the antibodies attached to these antigens; it is possible that this phenomenon is specific only in the sense that the antigen-antibody complex is specific.

All these articles add to the information which is accumulating about the reactivity patterns of cells derived from the lymphoid and reticuloendothelial systems. It is to be hoped that eventually they can be placed against a wider context to include consideration of the activities of mast cells, granulocytes and eosinophils, all of which can have non-specific more or less toxic activities.

interior of the supercontinent where they suppose that the influence of moisture-bearing wind currents could not reach. And no moisture means, of course, no glaciation. Similarly with coal deposits. Coal apparently can only form in areas where the minimum annual rainfall is 1,500–2,000 mm; and yet coal is found in the interior of Gondwanaland. The obvious conclusion then, according to Meyerhoff and Teichert, is that Gondwanaland could not have existed in the late Carboniferous and Permian. This contention is also supported by the presence of marine evaporites in Gondwanaland's interior within 15° of the Carboniferous and Permian poles. Although they do not say so explicitly, Meyerhoff and Teichert's position apparently conceals an anti-continental drift philosophy or at least one which does not allow ancient supercontinents.

It is the view of Meyerhoff and Teichert that a revolution in the concepts of southern hemisphere glaciation has been taking place simultaneously with the revolution in the Earth sciences but that the Earth scientists have been too busy to notice it—and thus one of the props of the new global tectonics has been whipped away. Clearly this is to overestimate the importance of glaciation in the continental drift context. Until Gough's recent article appeared the concept of the large Gondwanaland ice sheet was largely of historical interest, important at a time when evidence for drift was sparse but later superseded by far more convincing evidence. This apart, however, it would seem that the conflict between Meyerhoff and Teichert on the one hand and Gough on the other is essentially one of apparent irreconcilability of different disciplines—palaeontology and geology versus geophysics.

In his reply Gough wryly admits that "as a geophysicist, [he is] an outsider in these geological matters", but nevertheless proceeds to demolish his critics with the confidence of somebody who firmly believes in seeing wood for trees. Thus the existence of the thirty centres of glaciation in their respective positions leads him, with du Toit, to the interpretation that large ice sheets existed over part of Gondwanaland and not to Meyerhoff and Teichert's negative conclusion. The mountain-top ice caps and U-shaped valleys are easy to explain because if ice forms at all in a continent of rugged topography it will do so first on mountain tops. The resulting glaciers would thereby produce radial U-shaped valleys and, if rainfall and freezing continued, possibly continuous ice sheets. Thus the ice movement would be relatively inhibited such that few permanent traces of ice action would be left in the lowlands.

As for the question of rainfall in the

Gondwanaland interior, Gough takes the view that Meyerhoff and Teichert are formulating the problem the wrong way round. The starting point must be the existence of Gondwanaland, evidence for which is now overwhelming. The global tectonic evidence was not mentioned by Meyerhoff and Teichert. The presence of interior ice caps does not disprove the reality of Gondwanaland but only the idea that interior rainfall is impossible. The real problem therefore is to decide how, and under what conditions, the interior ice caps formed. In any case it is not clear to Gough that rainfall cannot occur far from continental margins as long as there is suitable topographical relief.

But there is a final irony. In his original article Gough considered for simplicity a continuous ice sheet 2 km thick and 3,000 km in diameter. The fact is that the same mass of ice distributed in lumps over the same area

will produce the same stresses except very near the surface. Thus a continuous ice sheet is not necessary at all! Moreover, an ice sheet 2 km thick and only 1,000 km in diameter will produce the same stresses in the lithosphere and the same stresses below its centre but at a lower depth. Thus the smaller sheet actually produces greater stresses in the asthenosphere where the tectonic flow takes place.

This whole argument, of course, is indicative of the sort of exchange which is likely to become increasingly common. The fact is that the revolution in the Earth sciences has been based almost completely upon geophysics, and many geologists feel that their discipline has not always received the consideration it deserves. The job now is to reinterpret several hundred years of geology in terms of the new global framework; and some friction is only to be expected.

Advances in Calcitonin Research

A RENAISSANCE in calcium metabolism research has taken place since the discovery of calcitonin nine years ago. Underlying this effort is the interest in the treatment of some fifty million people in the world today with bone-losing diseases. These diseases are loosely referred to as "osteoporosis".

The current investigative approach to the treatment of bone loss is the use of substances that inhibit bone resorption. The most popular of these is calcitonin, a hormone from the thyroid. Calcitonin acts directly and immediately on osteoclasts, the cells which resorb bone to inhibit their action. Because bone is constantly undergoing remodelling and replacement, prevention of bone loss, while new bone is continuing to be laid down, should lead to more bone. In next Wednesday's *Nature New Biology*, two letters contribute to the discussion of whether or not this hormone will prove of therapeutic importance.

The first of these contributions, by Anderson and his colleagues in Urbana, Illinois, reports the failure of moderately long-term treatment with porcine calcitonin to alter the retention of strontium-85. This isotope is unique in that its uptake in and release from bone is similar to that of calcium. Anderson *et al.* have measured whole body retention in dogs of this isotope, as a function of bone mineral turnover, and found that calcitonin did not delay its loss. The results are surprising in view of work done elsewhere (for example at the Hammersmith Hospital, London), which has demonstrated that calcitonin increases metaphyseal bone in rats and promotes positive calcium

in patients with Paget's disease. In contrast to both growing rats and Pagetic patients who have a rapid rate of bone turnover, bone turnover in normal dogs, however, is less and therefore more closely approximates the rates observed in osteoporosis in man. If the observations reported in next week's issue are true, it may be that calcitonin will not prove effective in preventing bone loss in patients whose rates of bone resorption are not abnormally high.

But in a second letter, Potts and his group at the Massachusetts General Hospital in Boston report that salmon calcitonin is 20–2,000 times more potent than the porcine hormone used in the previous study. Two explanations for this difference are tenable: either the salmon hormone has a greater affinity for receptor sites or it is more resistant to degradation. Potts *et al.* present evidence which supports the second explanation. As measured by radioimmunoassay, salmon calcitonin was found to have a slower rate of disappearance in the dog.

Salmon and porcine calcitonin differ in fifteen of their constituent amino-acids. That the hormones from different species have different rates of destruction introduces a new concept. This concept seems to be as follows: the activity of a peptide hormone is dependent not only on those amino-acids which confer biological activity but also possibly on other amino-acids which affect its rate of destruction. It follows from this that synthetic analogues which further delay degradation may lead to even more potent preparations than are found in nature.

Transport in the Phloem

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Exactly how nutrient materials travel through the green plant is still a matter for controversy. This review highlights some of the current points of interest.

MANY readers, not themselves botanists, will be aware of the old dictum "up the wood, down the bast". This draws attention to the two long-distance transport processes in higher plants: the movement of water up the wood from the soil supply to the transpiring leaves, and the independent movement of the products of photosynthesis down from the leaves to the roots. Strictly, this second movement, which is chiefly of sugars, occurs rather from regions of supply to regions of utilization, and so over any given portion of the plant axis is not invariably in the same direction. Broadly, however, the dictum is true. It is with the bast or phloem movement that this article is concerned.

The transport of sugars and other assimilates down the trunks of trees—to consider a concrete case—takes place in files of elongated cells making up the phloem. The most important of these cells are the sieve elements. These are living cells with very little content of living substance, disposed end-to-end like drain pipe sections. They are very narrow (typically about 20–40 μm in diameter), with a length about ten times as great as their width, and with the junction of one element and the next not unconstricted (as in earthenware drainage systems) but crossed by the fused end walls of the two elements. The transverse obstructions, which are about 1 μm thick, are pierced by numerous holes of diameter roughly 0.5–1 μm . For this reason they are aptly referred to as sieve plates, and the long files of cells which they cross at intervals of about 0.5 mm as sieve tubes. Down these tubes the assimilate stream flows at the relatively high velocity of about 50 to 100 cm per hour. Already it can be seen that here is a difficult problem in fluid dynamics, at least in the case of tall trees; but the problem has in the past fifteen years been made much more acute by the revelations of the electron microscope. Observations have shown that the most obvious content of the sieve elements (at least in the Angiosperms, and perhaps universally) is a fibrous protein, the P protein, which superficially resembles certain preparations of collagen or actin. Furthermore, this material appears in most careful preparations to occupy, fairly compactly, the pores in the sieve plates. If these plates constitute in themselves a considerable obstruction to flow, it is easy to see the problem which arises when their pores appear densely occluded with fibrous material (Figs. 1 and 2).

The discoverer of sieve tubes (Hartig, 1837, 1860) suspected that they were the conducting channels and although controverted, this view has steadily gained ground until today it is almost unchallenged. Recently autoradiography of thin sections has been the most potent factor in the strengthening of this view. Starting with the earlier work of Biddulph¹ using ³⁵S and ³²P, improved resolution has been obtained, first with ¹⁴CO₂ incorporated naturally into assimilates, and then with

tritiated sugars applied externally to the leaf^{2–4}. The difficulty of the technique arises largely from the water solubility of the compounds concerned, but this has been overcome to such an extent that very recently⁵ natural ¹⁴C-assimilate has been autoradiographed in resin-embedded material cut no thicker than 1 μm . In both the tritiated material (cut at 6–8 μm in paraffin wax) and this ¹⁴C material, convincing evidence has been presented that transport is confined to the sieve tubes. Incidentally, labelled and unlabelled sieve tubes occur in juxtaposition², suggesting that to some extent they act independently.

Rate and Pattern of Movement

Recent work by Zimmermann⁶ has confirmed the generally accepted view of the velocity of transport. The alternation of day and night would be expected *a priori* to produce a rhythmic change in the transport of assimilates, and Huber *et al.* (1937) measured a sinusoidal variation with height in the sugar concentration in the phloem of trees and were thus able to derive a velocity of movement; it was three or four times larger than currently accepted values. Zimmermann reasoned that changes in water potential in the xylem (which is very close to the phloem) would independently affect the sugar concentration. In certain trees he has demonstrated that sucrose, the most usual sugar found in the transport stream, undergoes in the

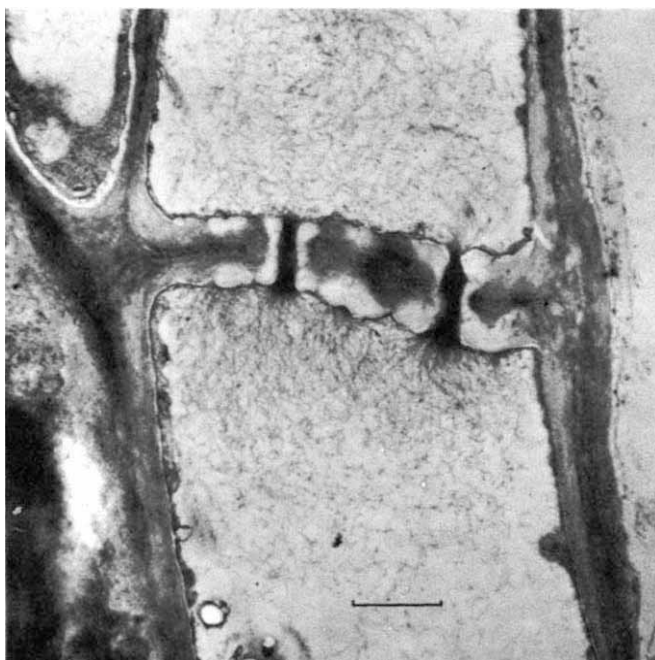


Fig. 1 Longitudinal section of a sieve tube of *Helianthus annuus* showing sieve plate. Fibrils of P protein occupy the lumens and densely occlude the pores. By courtesy of A. W. Siddiqui. (Index line 1 μm .)

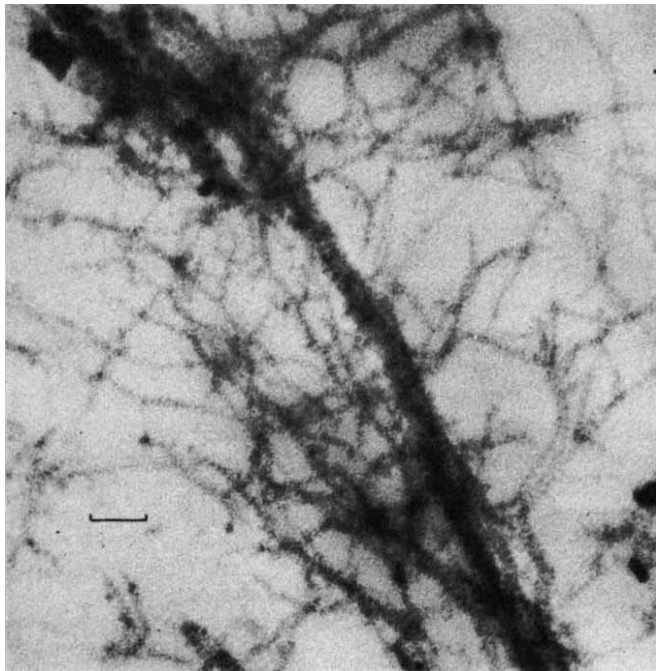


Fig. 2 Banded fibrils of P protein from sieve tube of *Salix caprea*. Note that the bands tend to align themselves transversely. By courtesy of U. Mishra. (Index line 0.1 μm .)

sieve tubes some degree of inter-conversion with the higher sugars raffinose and stachyose. By measuring at various heights simultaneously the ratios between these three sugars he showed that they varied sinusoidally. The velocity of movement implied by these "ratio waves" (30–70 cm h^{-1}) fits well into the accepted range.

On the disputed question of whether or not the sieve tubes are able to conduct in both directions at once (certain suggested mechanisms would allow them to), the past two or three years have provided evidence but no settlement. Eschrich⁷ in 1967 reported work on broad bean in which ^{14}C naturally assimilated by one leaf and fluorescein artificially applied to another both appeared in the exudate from an aphid feeding on the stem between them. Because aphids are known to tap single sieve tubes, the evidence was strong for bidirectional movement. But Eschrich himself noted that another explanation was possible: opposite unidirectional movement in parallel sieve tubes with lateral transfer between them. Ho and Peel⁸, using ^{14}C and tritiated sugars in willow segments, obtained rather similar results, as did Trip and Gorham⁹ using microautoradiography instead of aphids to localize the tracers. But strong contrary evidence turned up in the work of Peterson and Currier¹⁰. Applying fluorescein at the mid-point of an internode and allowing only a short period (one hour) for translocation, they found on sectioning the stem above and below the mid-point that the dye had moved both upwards and downwards, but only in different bundles. When a longer time was allowed for translocation, however, single bundles were labelled in both directions, almost certainly as a result of the lateral movement of the tracer at the nodal anastomoses. Reluctantly, it must be recognized that this crucial problem, on which considerable effort has been expended, is still unresolved.

Factors affecting the Rate

Although inhibitors have long been used for investigating physiological processes, they have provided a singularly unsatisfactory picture of translocation, and the past few years have confused it rather than otherwise. The chief difficulties arise from the fact that it is hard to discriminate between the transport process itself and the terminal processes in which assimilates are loaded into or unloaded from the conducting channels. In addition the proximity of the xylem, itself capable

of long distance conduction, and the propensity of the sieve plates for rapidly sealing themselves off with callose, constitute further difficulties in interpreting inhibitor results in such a way as to decide unambiguously between mechanisms. Harel and Reinhold¹¹ ensured in soyabean that the vein-loading process was not involved by cutting off the ^{14}C -treated leaflet before the introduction of the inhibitor dinitrophenol (DNP). They found, however, that not only was the transport not inhibited, but downward movement to the roots was promoted. This evidence contradicts previous conclusions. In a well argued case the authors interpret their results as support for the Münch hypothesis (discussed later), though it is not clear how the opening of sieve tubes at their upper ends could leave the pressure gradient (on which the Münch theory relies) unimpaired; nor how they decided against movement throughout the xylem to the roots, a distinct possibility with the likely disturbance of root function through DNP and an overhead water supply. It is an indication of the complexity of the problem that at least two other factors need to be considered before the results can be interpreted confidently—the vascular anatomy, and the effect of the treatment on sieve plate callose. For strangely, in certain circumstances the laying down of callose can accelerate transport¹², a possible consideration in Harel and Reinhold's results.

Low temperature is another factor which has been investigated fairly recently. A striking feature of the phenomenon is that it only requires cooling of a short length of axis (say 2 cm) to reduce drastically the mass transfer rate¹³. Furthermore, if the low temperature is maintained, the subsequent behaviour of the plant varies. With plants acclimatized to cold regions the rate may recover to its previous value fairly quickly, whereas with plants from warmer regions it remains low. In the latter case, restoration of the cooled portion to ambient temperature is followed by recovery in mass translocation rate after a certain time. Geiger considers possible mechanisms for these effects. With naturally cold climate plants the recovery in rate when the axis is held at a low temperature (0.5° C) takes no longer when 8 cm of axis is cooled than when 2 cm is; he argues that this tells against the recovery being due to a build-up of the concentration differential over the cooled region, presumably because a bigger differential would be required over the longer region, and this would take longer to establish. But over the small distances involved and with sugar transport as large as it is, this argument is not so cogent as it seems at first.

It would be natural to suggest that the slow-down caused by chilling is due to a decrease in the rate of the metabolic energy supply. This interpretation would favour the electro-osmotic theory, since unlike the Münch hypothesis its energy supply is mediated all along the route, and not merely at the ends. Geiger, however, suggests an alternative explanation: that the resistance of the transport channels is raised by the chilling process by changes in the sieve tube organelles. But a ten-fold increase is required, and it seems unlikely that the sparse contents could manage this unless the changes took place in the vicinity of the sieve plates. Additional callose, and a denser plugging of the pores with P protein, seem obvious claimants for investigation. It remains possible, however, that the primary cause is a decrease in the rate of supply of metabolic energy. The electro-osmotic theory would then explain recovery in terms of a re-adjustment of the pore mechanism.

Sieve Tube Contents

The contents of the sieve tubes have remained a source of interest, especially those elements which can be considered part of the mechanism, rather than part of the translocation stream. The content of ATP, already known to be fairly high from the work of Kluge and Ziegler¹⁴, has been confirmed using aphid stylet extract of willow¹⁵, and more recently by measurements on the copious phloem exudate of *Yucca inflorescence*¹⁶. All reports give a fairly high concentration

(0.6–1 mg ml.⁻¹ or about 0.002 M). No doubt this is significant for the callose closing reaction, and perhaps also for the flow mechanism. Phosphatases have been further reported on. In view of the slightly alkaline reaction of sieve tube sap the incidence of alkaline phosphatase in mature *Pisum* sieve tubes¹⁷ is interesting, though most of the reports have been of acid phosphatases. Zee¹⁸ found no acid phosphatase activity in the "slime" of *Pisum* (contra a report¹⁹ for *Tilia*) though there was activity in the plasmalemma and endoplasmic reticulum of mature tubes. Robards and Kidwai²⁰ reported ATPase activity at pH 7.2 in immature phloem and ray cells; lead deposits occurred on both the inner and outer edges of the plasmalemma as well as elsewhere. Yapa (unpublished) has found ATPase activity at pH 7.2 in the plasmalemma of mature sieve tubes.

But of all the sieve tube contents the one of major interest has continued to be the famous slime, now appropriately renamed P protein²¹. Its proteinaceous nature has been inferred from staining reactions, ultrastructural appearance, enzyme digestion (Yapa, unpublished results) and chemical analysis of phloem exudate²², and there seems little doubt that the banded fibrillar material so often seen in electron micrographs is pure protein, probably of a rather chemically resistant nature. It seems to be the case that ontogenetically it originates from "slime bodies" often composed of bundles of more or less parallel tubular elements, resembling microtubules but distinct from them²³. There seems to be some diversity in the tubular-type slime bodies, and the slime bodies may in some plants be composed of fibrils from the first²⁴. The sieve tubes may be the sites of P protein production for some time after maturity sets in; and P protein, or something similar to it, may be produced in ways other than from typical slime bodies. For example, it may come in addition from protein crystals in Leguminosae²⁵ and from sieve tube plastids in certain Caryophyllales^{26,27}. In primary tissue the P protein seems to be connected with peculiar cell organelles called "spiny vesicles"²⁸. Later studies seem to have confirmed this idea²⁹. If so, then it would seem to be the case that in protophloem, adjacent parenchyma cells assist in the production of P protein for the sieve elements. Furthermore, the occurrence of typical P protein in the plant kingdom seems to have been extended more firmly to the monocotyledons³⁰, so that when everything is considered, the conviction that this

material fulfils some essential role in the translocation process has been strengthened.

Of the other constituents of the sieve tube I shall mention only the endoplasmic reticulum (ER). Recent studies have confirmed this to be a fairly abundant element of the cytoplasm in mature tubes³¹. It is frequently present in parietal "stacks", with the constituent membranes lying either parallel to the tube wall or set almost at right angles to it (Fig. 3). Often it occurs in curious convoluted configurations, like papier-mâché egg boxes stacked within one another³². It usually seems to be anchored closely to the wall, and sometimes when the membranes stand perpendicular to the latter they seem to be continuous with dark lines within it. Certainly the thick sieve tube wall, the cellulose fibrils of which probably run in shallow spirals and which seem to contain little cementing substance, often has pronounced radial striations in longitudinal section. These appearances have led to the suggestion³³ that the ER stacks are really plasmalemma which in the turgid cell is buried in the cellulose wall, but which emerges more or less rapidly (hence the variety of configurations) into the lumen on collapse of turgor. Whether this unorthodox idea can be substantiated remains to be seen. Attempts to fix and section plasmolysed sieve tubes have not so far yielded supporting evidence.

The Question of Mechanism

In this short review I shall consider only two mechanisms (though others are being canvassed); the Münch or pressure flow hypothesis³⁴, which considers movement in the sieve tubes to be due to a gradient of hydrostatic pressure; and the electro-osmotic hypothesis, which invokes electrical forces called into play at the sieve plates by the active secretion of potassium ions^{33,35}. Both involve mass flow, and both therefore have a similar stake in the observation (or otherwise) of simultaneous bi-directional movement, which was discussed inconclusively earlier. One important issue on which the theories differ is in their relation to the state of the pores in the sieve plate. If the natural functioning condition of these is as Fig. 1 indicates, then simple calculation shows that the pressure gradient required by the Münch hypothesis is far too great to be realizable in practice—of the order of several hundred atmospheres per metre³⁶. On the other hand, there is no problem here for the electro-osmotic theory—it positively requires that the pores be occluded in some such way as the micrograph shows. On the other hand, this theory runs into difficulties of its own, particularly that it seems to imply a very high current density for potassium ions, perhaps as high as 1 A cm⁻² of sieve tube cross-section. In this connexion the suggestion of a greatly expanded plasmalemma in the sieve tube is important, for secretion of the ion across this membrane would be the resistive as well as the energy-transducing step.

The conflict between the two hypotheses centres largely on whether micrographs such as Fig. 1 are faithful representations of the functioning sieve plate. It must be emphasized that the sieve tubes have high turgor pressure coupled with relative ease of longitudinal movement; thus any attempt to cut into them or manipulate them for examination in the electron microscope runs the risk of causing violent axial surges. P protein being present in the lumen, such surges will arguably carry it to and through the sieve plates. The supporters of the Münch hypothesis argue that the pores in the functioning tube are unoccluded with P protein. Fig. 1 accordingly shows an artefact; it is a difficult matter (though micrographs have been obtained) to record faithfully the functioning condition. That is why the pores so rarely appear open. The supporters of the electro-osmotic theory take a different view. They maintain that much of the evidence adduced to support the "artefact" view in fact does almost the opposite. Even the very narrow and exposed sieve tubes of small leaf veins, so narrow that the sieve plates may have only one pore, show the same sort of plugging³⁷. The same can be said, they point

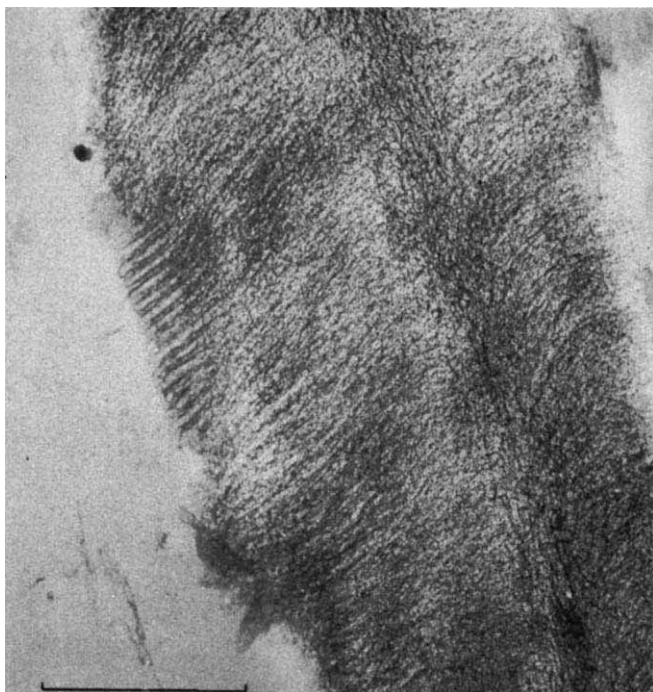


Fig. 3 Membrane stacks in close contact with the sieve tube wall in *Nymphoides* petiole. By courtesy of R. L. Jones. (Index line 0.5 μ m.)

out, of the short curved sieve tubes of callus tissue³⁸. Of course, every device of chemical or physical manipulation (such as freeze drying, freeze etching and prior wilting or boiling) has been pressed into service to resolve the issue, but neither side seems disposed to give way^{39,40}.

It is often urged that the use of macroscopic equations of viscous flow is misleading in the case of channels which might be no more than 100 Å wide, so that the argument about the state of the pores is for the moment pointless. But some evidence indicates that in very fine channels the viscosity of water does not drastically fall; on the contrary it seems to rise. Churayev, Sobolev and Zorin⁴¹ found an increase of 40% in quartz capillaries of 0.04 mm radius. Thus the problem is a real one.

The special evidence for the Münch hypothesis is not particularly strong. It turns mostly on its simple physical character. Also, it can allow the ready transport in sieve tubes of large particles like viruses⁴². On the other hand, the electro-osmotic theory has numerous arguments of an indirect nature to recommend it. It is much more physiological, and can take in its stride any well authenticated evidence for an effect of poisons, anoxia or low temperature. It offers a major role for potassium, hitherto without one; and it also makes substantial suggestions for the functions of P protein, membrane stacks, companion cells and the thick sieve tube wall. More direct (though hardly decisive) is the experimental evidence for a saw-tooth profile of electrical potential⁴³ in the sieve tubes, as required by the theory; and that potassium deficiency too small to cause visible symptoms apparently has a primary effect in lowering translocation⁴⁴.

Two recent approaches to the investigation of mechanism should be mentioned. Fensom and his collaborators⁴⁵ have applied Nomarski interference optics to the cinematography of functioning *Heracleum* phloem. No actual translocatory movement of particles was visible across the sieve plate, but an unusual type of particle movement (probably of starch grains) was reported. But in view of the reports that the release of starch grains from plastids in sieve tubes is probably an injury effect one needs evidence that the sieve tubes recorded were functioning normally. Milburn⁴⁷, in what he described as a "lunatic fringe" experiment, followed up a clue from Bose and tried the effect of simple massage (squeezing gently between finger and thumb) on the stem of young plants of *Ricinus*. Repeated at intervals three times a day for several days, this had the remarkable effect that when an oblique incision was made into the bark the phloem exuded copiously, up to 1 ml. of sap being obtainable from a single incision in a plant 20 cm tall, with little progressive decrease in concentration. Milburn interprets this phenomenon as follows. The sieve plate pores are normally open. Massage triggers an automatic sealing mechanism which operates to block the pores (and so serves in severed plant organs a function analogous to blood clotting). Subsequently an anti-sealing mechanism frees the pores and enables translocation to re-start. Repeated massage exhausts this double reaction so that when an incision is finally made the plant is defenceless and bleeds phloem sap profusely. Milburn rejects the obvious suggestion that massage cumulatively disrupts a natural state of affairs illustrated by Fig. 1, on the grounds that if a plant which has been induced to bleed profusely is massaged at the point of exudation, the exudation stops within minutes or seconds. Temporary application of pressure 2.5 cm from the incision on both sides acts similarly. My interpretation would be, on the contrary, that the whole phenomenon supports the *prima facie* understanding of Fig. 1. Massage ejects the P protein plugs from the sieve pores, and repeated massage prevents their re-establishment and furthers the dislocation. In the rapid exudation following subsequent incision the disorganized P protein is being hurried through the sieve plate pores; temporary slackening of the flow by finger pressure enables it to build up in front of one or more sieve plates so that on release an effective blockage remains. This interpretation seems less contrived than invoking a sealing-unsealing mechanism of

unspecified character which, thrown out of gear by massage, can nevertheless recover sufficiently when exudation is in full operation to respond to pressure of the fingers.

Future Developments

Taken by itself it is hardly disputable that the electron microscope evidence supports the view that the sieve plate pores are plugged in a carefully structured fashion with P protein fibrils. The difficulty of reconciling this picture with the physiological facts leads to the impasse. Thus after more than a century of investigation there is no agreed answer to the question of how the sieve tubes conduct. What developments may be expected in future work on the problem? On the anatomical side we may anticipate a wider range of comparative studies with the electron microscope, including more emphasis on perennial monocotyledons and the lower vascular plants, and on freeze etching and other recent techniques. The sieve tube wall will probably be examined carefully. On the biochemical side, the chemical and physicochemical characterization of P protein will be an obvious target, and also the nature of the callose-triggering reaction and the metabolic function of high energy compounds in the sieve tubes. Microelectrode techniques and the movements of potassium will certainly fit into the picture. Finally, on the level of whole plant physiology, quantitative data for the movement of tracers of different kinds will probably be combined with computer analysis of more adequate model systems. With patience we may hope to see fuller light on this intriguing phenomenon.

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The Space Scars of Earth

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There is some evidence for a meteoritic origin of craters in the Earth's surface. In this article the characteristic features of impact scars are discussed with the help of some examples.

THE two planetary bodies whose surfaces can be seen best from Earth and from spacecraft are the Moon and Mars. A dominant characteristic of both surfaces is a complex of circular craters of widely varying size and there is now considerable evidence that most of these craters resulted from the infall of the smaller, and less massive, bodies of the solar system—asteroids, meteoroids and possibly comets. We see the primary and secondary impact scars of these collisions with material from interplanetary space. It can be argued that the Earth should have suffered a similar celestial bombardment, and evidence of this is now growing rapidly, but because the Earth's surface is subject to many changes initiated by the atmosphere and oceans, the impact craters often have to be untangled from a maze of other features that may hide them from all but the persistent investigator.

Increasing Attention

It is rather surprising that in the past the detection of impact scars on Earth attracted little attention from most geologists. This situation is now changing rapidly and new information is becoming available in such profusion that any review of the subject rapidly becomes out of date.

Near the end of the last century there were tentative suggestions that the formation of certain depressions on Earth might have a connexion with meteoritic impact. G. K. Gilbert, for example¹, discussed this possible origin for the crater in Arizona—then known as Coon Butte—and for Lonar Lake in India. He discarded the hypothesis, chiefly because of a lack of understanding of the tremendous explosive force inherent in an impact at typical interplanetary collision speeds (15 to 25 km s⁻¹).

The scientific study of terrestrial impact features can properly be dated from the presentation of two papers^{2,3} before the Academy of Natural Sciences in Philadelphia on December 5, 1905. Both Barringer and Tilghman put forward in detail numerous reasons why Coon Butte should be considered a meteoritic crater and not the result of volcanic action or a steam explosion. In the face of considerable opposition to his theory, Barringer followed up his original paper with a continuing study of the Arizona crater until his death in 1929; in recognition of his pioneering work this feature is now officially named the Barringer Crater. (Barringer also has a crater on the Moon named after him.)

It was not until 1926 that the crater at Odessa, Texas^{4,5}, was suspected to be of meteoritic origin and in the next few years impact features were recognized in a number of other sites⁶. Spencer⁶ reviews accounts of twenty-eight craters in seven locations, all of which are now considered to be meteoritic in origin and to contain associated meteoritic material. Spencer also mentioned the Tunguska event in Siberia on June 30, 1908, but this is not generally considered to have produced recognizable impact craters on the Earth's surface⁷.

Cryptovolcanism

The second phase in the study of terrestrial impact scars commenced with a series of papers⁸⁻¹⁰ by J. D. Boon and C. C. Albritton who gave reasons why certain geological structures, previously called cryptovolcanic, should be considered of impact origin. In this case the argument was not as direct as for the craters listed by Spencer. The ages of the cryptovolcanic features were measured in hundreds of millions of years so that little remained of the original form, and any meteoritic material that might have been scattered in the area had long since disappeared. A common characteristic of these generally circular structures is the evidence of strong explosive action at a period that did not coincide with the age of the rocks involved. A wide acceptance of the views of Boon and Albritton came only after recent advances in the knowledge of highly shocked minerals. Eight of the nine structures listed by these authors as possibly meteoritic are now generally accepted as impact scars.

Canadian Studies

The third phase in the study of meteoritic craters did not start until 1950 when V. B. Meen of the Royal Ontario Museum led an expedition to Northern Ungava, Quebec, to investigate a circular lake that seemed to be a geological anomaly in the peninsula¹¹. This feature, now named the New Quebec Crater, was pronounced meteoritic by Meen. Other discoveries among the ancient rocks of Canada were made soon afterwards, many as a result of a systematic search of air photographs organized by C. S. Beals¹² at the Dominion Observatory, Ottawa. Beals also introduced a new technique in the study of these ancient structures; various geophysical measurements were carried out systematically in a coordinated programme and then combined to give an overall picture. A good example is the investigation of the Brent Crater¹³, which included aerial photography, topographical survey, surface geology, deep core drilling, and surveys by magnetic, seismic and gravity techniques. Brent has been used as a type example for comparing and assessing data secured in various ways. About twenty Canadian features are now being studied by these comprehensive geophysical methods¹⁴.

The latest developments in the study of meteoritic impact features have arisen from the recognition of several definitive

Table 1 Identification Criteria for Impact Craters

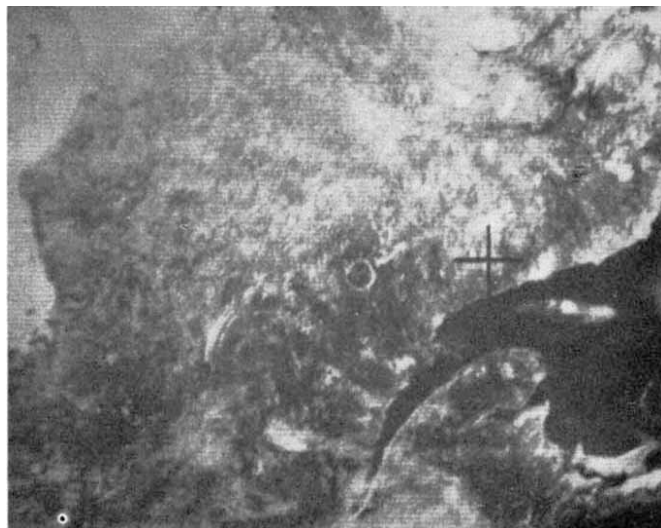
Form and structure	General circular form, depression with raised rim, radial fracturing and rim strata uplift, central uplift.
Meteoritic material	Presence of meteorites, nickel-iron spherules, iron shale of meteoritic origin.
Rock breccia	Impact produced breccia—on surface with fragments approaching metre sizes or larger, in drill cores with fragments of centimetre sizes, in rock samples as micro-breccia or suevite.
Shatter cones	Cone shaped formations found in a variety of rock types.
Mineralogical shock evidence	Numerous small scale and microscopic characteristics ranging from planar features and deformation discontinuities to fused glassy fragments with vesicles and streaks, forming a pumice-like material with lechatelierite.
Impact melts	Igneous materials—rocks, lavas, glasses, formed by impact melting.

characteristics, both microscopic and macroscopic, of highly shocked materials. As early as 1946 Dietz¹⁵ pointed out the value of shatter cones as shock indicators and since 1960 further knowledge of shock metamorphism has accumulated rapidly¹⁶ as a result of X-ray analysis and work with the microscope and the electron microprobe. The application of these new techniques to samples recovered from suspected impact sites has made it much easier to distinguish between structures that have only a superficial resemblance to impact craters and those that are genuine impact scars.

Geophysicists, geologists and astronomers now have at their disposal a wide variety of criteria for deciding the origin of a possible terrestrial impact feature. Some of the more important of these are shown in Table 1 but the list is by no means exhaustive. The overall picture of any site and the possibilities of origins other than impact must be considered carefully in coming to a conclusion in any given case.



Fig. 1 The Barringer Crater, Arizona, diameter 1.2 km, photographed from the north near local noon on January 29, 1967. The polygonal form of the crater rim, seen in so many lunar craters, is quite evident.



[Photo: Canadian Meteorological Service]

Fig. 2 The Manicouagan Crater, Quebec, diameter 60 km, photographed from the ESSA-8 satellite on March 18, 1969. The crater appears at centre while a much larger feature, the Hudson Bay arc, is at upper left. This arc is still on the list of suspected impact scars³⁴.

Energy Release

The shape of a newly formed impact crater is caused by the sudden release of the kinetic energy of the impacting mass within a small volume somewhat below the original impacted surface. Matter will be ejected approximately equally in all directions and the impacting meteoroid will be broken up, pulverized and partially vaporized in the process. The result is a bowl shaped depression with a raised rim and, in some cases, a central uplift. Typical energies range from $\sim 10^{20}$ erg for a crater of diameter about 1 km to $\sim 10^{30}$ erg for a diameter of 50–100 km.

The form of relatively recent terrestrial impact craters corresponds closely with the youngest craters on the Moon and craters produced by artificial explosions on the Earth. The best example of a comparatively fresh impact scar is the Barringer Crater (Fig. 1) which has been preserved by the arid climate of Arizona from excessive erosion and sedimentation during the 30,000 or more years since it was formed. The outward tilting of the normally horizontal rock strata is very evident, even to the casual observer, and one-sixth or more of its original depth has probably been filled up. In the case of the much older Canadian craters in the same size range, the original depression has often been completely filled by sediments tens or hundreds of millions of years old. Geophysical methods introduced in the 1950s make it possible to outline the original crater depth and the brecciated volume beneath the crater floor in these instances. The old craters of a category in which the original diameter was more than several kilometres normally show a central uplift. In many cases, the original crater has been filled and the rim eroded, but the central uplift is the one dominant feature which remains clearly visible. Some details of the central uplift in terrestrial craters are not yet completely understood but, in broad terms, the uplift may be ascribed to a combination of post-impact slumping of the crater walls, isostatic adjustments, and elastic rebound in the subsurface rock layers¹⁷. The mechanisms of central uplift will have a greater effect in the larger craters, since here the energy of impact is in a higher ratio to the energy required to deform the geological strata. One of the large Canadian impact scars, Manicouagan (Fig. 2), demonstrates well the peripheral trough which is often the most conspicuous structural feature in deeply eroded ancient craters.

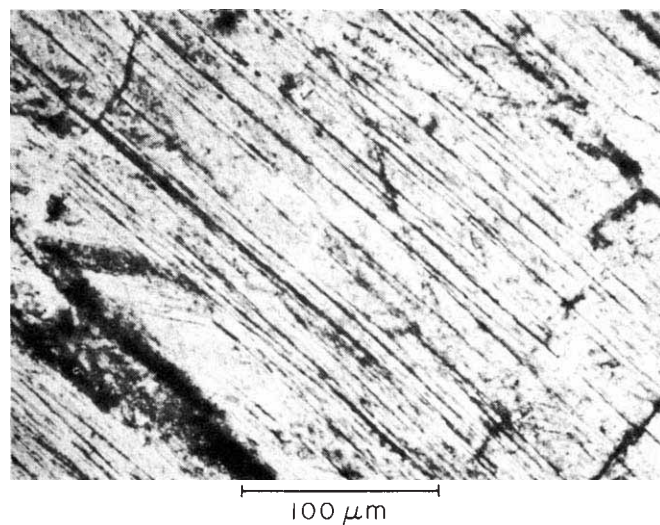
Meteoritic Evidence

The presence of meteoritic material was once considered essential for the positive identification of an impact crater, and meteorites have been associated with all the smaller and younger impact features. Nickel-iron spherules of several types in the millimetre size range were first found in 1946 by Nininger^{18,19} at the Barringer Crater, and have been found more recently at a number of sites where meteorites have not yet been located. The manner of formation of these spherules is still a matter for discussion but there is no doubt that they are a product of the original meteoroid²⁰. In the case of the very old impact features identifiable meteoritic material has not often been found. Calculations of the diameters of the meteoroids producing these craters have varied from 1/20 to 1/30 of the crater diameter for a stony meteoroid, so the meteoroid volume would have been much less than 10^{-4} times the volume of terrestrial rock involved. Surface meteoritic material would not have remained *in situ* for millions of years, and probably only the nickel-iron portion of this material would be identified in the drill core sampling. Only a very small fraction of the total volume is studied in drill core methods and it is not surprising that there is little direct evidence of the original meteoroid.

Evidence of the shattering force of space impact may be seen in the broken rock fragments commonly associated with space scars. Barringer² called early attention to the large-scale debris scattered around the crater that now bears his name. Drill cores, such as those from the Holleford Crater²¹, illustrate vividly the type of rock breccia that is found below the crater floors. Breccias of an even finer grain size can be identified with a microscope²².

The work of R. S. Dietz on shatter cones has already been mentioned and he has recently reviewed their occurrence at terrestrial impact sites²³. These cone-shaped structures are formed by shock in a variety of rock types at pressures from 20 to 100 kbar, much greater than the pressures usually ascribed to terrestrial volcanic mechanisms.

Other mineralogical shock evidence is relevant to even higher pressures²⁴ and at shock pressures between 100 and 300 kbar planar features (closely spaced fine parallel lines) develop in quartz, plagioclase and other minerals. An example of this microscopic structure in quartz is shown in Fig. 3. Apart from the planar features there is other evidence of deformation, for example, slip bands, kink bands and deformation



[Photo: M. R. Dence]

Fig. 3 A thin section of quartz from the central uplift of Lake St Martin, Manitoba, showing one dominant set of planar features and several other faint sets.

lamellae. At pressures from 300 to 500 kbar there are still planar features and also the highly shocked phases of SiO_2 , coesite and stishovite. In this pressure range undisturbed

Table 2 Locations of Impact Sites

North America	Canada	18
	United States	14
South America		2
Europe		10
Asia		3
Africa		8
Australia		7
Total		62

Table 3 Selected List of Terrestrial Impact Sites

	Latitude	Longitude
Aouelloul, Mauritania	21° 15' N	012° 41' W
Barringer, Arizona	35° 02' N	111° 01' W
Bosumtwi, Ghana	06° 32' N	001° 23' W
Boxhole, N. Territory, Australia	22° 37' S	135° 12' E
Brent, Ontario, Canada	46° 05' N	078° 29' W
Campo del Cielo, El Chaco, Argentina	27° 28' S	061° 30' W
Carswell, Saskatchewan, Canada	58° 27' N	109° 30' W
Charlevoix, Quebec, Canada	47° 32' N	070° 18' W
Clearwater, Quebec, Canada	56° 10' N	074° 20' W
Crooked Creek, Missouri	37° 50' N	091° 23' W
Dalgaranga, Western Australia	27° 45' S	117° 05' E
Decaturville, Missouri	37° 54' N	092° 43' W
Deep Bay, Saskatchewan, Canada	56° 24' N	102° 59' W
Dellen, Sweden	61° 50' N	016° 45' E
Flynn Creek, Tennessee	36° 16' N	085° 37' W
Gosses Bluff, N. Territory, Australia	23° 48' S	132° 18' E
Haviland, Kansas	37° 37' N	099° 05' W
Henbury, N. Territory, Australia	24° 34' S	133° 10' E
Holleford, Ontario, Canada	44° 28' N	076° 38' W
Howell, Tennessee	35° 15' N	086° 35' W
Jänisjärvi, Karelia, USSR	61° 58' N	030° 55' E
Jeptha Knob, Kentucky	38° 06' N	085° 06' W
Kaalijärvi, Estonia, USSR	58° 24' N	022° 40' E
Kentland, Indiana	40° 45' N	087° 24' W
Köfels, Austria	47° 13' N	010° 58' E
Lac Couture, Quebec, Canada	60° 08' N	075° 18' W
Lappajärvi, Finland	63° 10' N	023° 40' E
Liverpool, N. Territory, Australia	12° 24' S	134° 03' E
Lonar, Maharashtra, India	19° 59' N	076° 34' E
Manicouagan, Quebec, Canada	51° 23' N	068° 42' W
Manson, Iowa	42° 35' N	094° 31' W
Middlesboro, Tennessee	36° 36' N	083° 36' W
Mien, Sweden	56° 25' N	014° 55' E
Mistastin, Newfoundland, Canada	55° 53' N	063° 18' W
Monturaqui, Chile	23° 54' S	068° 18' W
New Quebec, Quebec, Canada	61° 17' N	073° 40' W
Nicholson, NWT, Canada	62° 40' N	102° 41' W
Odessa, Texas	31° 48' N	102° 30' W
Pilot, NWT, Canada	60° 17' N	111° 01' W
Pretoria Salt Pan, South Africa	25° 30' S	028° 00' E
Ries, Germany	48° 53' N	010° 37' E
Rochechouart, France	45° 50' N	000° 56' E
Roter Kamm, South West Africa	27° 45' S	016° 17' E
St Martin, Manitoba, Canada	51° 47' N	098° 33' W
Serpent Mound, Ohio	39° 02' N	083° 25' W
Sierra Madera, Texas	30° 36' N	102° 55' W
Sikhote-Alin, Siberia, USSR	46° 07' N	134° 40' E
Siljan, Sweden	61° 05' N	015° 00' E
Skeleton, Ontario, Canada	45° 15' N	079° 27' W
Steen River, Alberta, Canada	59° 31' N	117° 38' W
Steinheim, Germany	48° 02' N	010° 04' E
Strangways, N. Territory, Australia	15° 12' S	133° 35' E
Sudbury, Ontario, Canada	46° 36' N	081° 11' W
Talemzane, Algeria	33° 18' N	004° 06' E
Temimichât, Mauritania	24° 15' N	009° 39' W
Tenoumer, Mauritania	22° 55' N	010° 24' W
Vreddefort, South Africa	27° 00' S	027° 30' E
Wabar, Saudi Arabia	21° 30' N	050° 28' E
Wahnapitae, Ontario, Canada	46° 44' N	080° 44' W
Wells Creek, Tennessee	36° 23' N	087° 40' W
West Hawk, Manitoba, Canada	49° 46' N	095° 11' W
Wolf Creek, Western Australia	19° 18' S	127° 47' E

mineral crystals begin to change to the disordered or amorphous phases and at 500 to 650 kbar glasses containing vesicles and streaks are formed and most of the original texture of the crystalline rocks is destroyed. At pressures approaching 1,000 kbar and temperatures of 5,000 K complete melting occurs.

High temperatures are developed in an impact event, particularly the larger terrestrial features. The surface rocks melt and leave igneous materials in the form of glass in mixed breccias, layers of rock melt, and intrusions into basement rocks below the crater. Dence²⁵ has noted these melts at about forty impact sites.

General Characteristics

There is no single standard listing of terrestrial impact craters and a number of lists (refs. 26–33 and R. W. Barringer, personal communication) have, for example, been published since 1960. Many suspected sites are now known at which a full scale field study has not yet been possible and most of these are therefore in the doubtful category at present. To illustrate some general characteristics of the Earth's impact scars, I have selected from these lists and from more recent literature 62 sites which include the best authenticated examples of impact craters. Preference has been shown in this selection for evidence of high shock effects. The 62 sites include 112 individual craters with diameters ≥ 10 m, an arbitrarily chosen lower size limit. The geographical distribution of the 62 sites is summarized in Tables 2 and 3.

Estimates can be made for the original diameter of each crater and for its age. Only small accuracy can be achieved with individual craters, particularly when their age is large, but the overall statistical picture is probably free from serious systematic errors. Diameter and age estimates for the 112 craters have been plotted in Fig. 4—the eight groups of multiple craters have been separately identified.

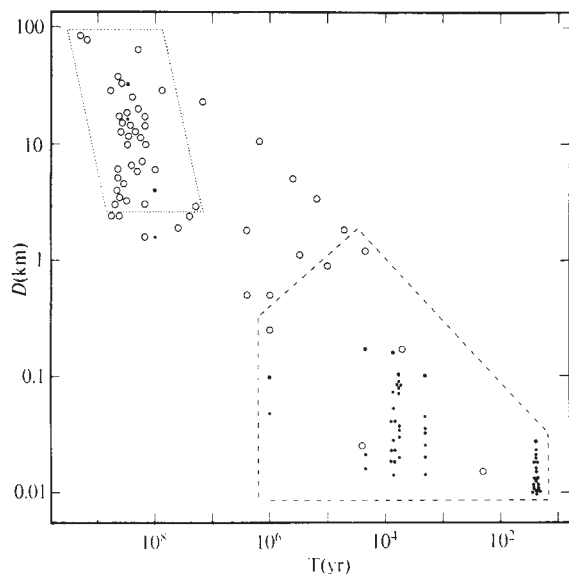


Fig. 4 A logarithmic plot of the estimated original diameter of each crater, D km, plotted against the estimated age, T yr. Meteorites have been found associated with all craters in the area enclosed by dashed lines. Central uplift is present in three-quarters of the craters in the area enclosed by dotted lines. ○, Single crater; ●, crater group.

One must be careful not to draw unwarranted conclusions from Fig. 4. It has been plotted more to show the distribution in age and size of the recognized impact features on Earth, than to demonstrate any important relation. Even though the logarithmic scale tends to crowd points to the left of the diagram, the apparent lack of large recent craters is probably a real effect; massive space impacts were undoubtedly more

common when the solar system was younger. The lack of old small craters is caused by erosion and obliteration.

If the assumption is made that the majority of large lunar craters were formed by impact, it is natural to compare the numbers found per unit area on the Moon with the numbers found on Earth, as both Moon and Earth have been subjected to the same interplanetary environment. An approximate count indicates that the crater density on the Moon is several orders of magnitude greater than it is here but the impossibility of estimating the terrestrial obliteration effects over hundreds of millions of years makes a quantitative comparison almost useless. It can safely be said, however, that lunar crater statistics suggest that many more terrestrial impact features will be found if we look hard enough for them. At a time when a very large effort is being expended on the study and interpretation of lunar surface materials, the analysis of the corresponding materials from terrestrial impact sites is an essential concomitant activity.

I acknowledge many profitable consultations with Mr M. R. Dence.

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Circular Polarization: Jupiter and Other Planets

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Circular polarization of scattered light from Jupiter has now been studied during a recent opposition of the planet, showing changes in the sign of the north and south polar values indicative of a non-magnetic origin for the polarization. Preliminary observations on Mars, Venus, Mercury and the Moon demonstrate the generality of this effect and its potential usefulness.

SINCE our discovery of circular polarization in the scattered light from Jupiter¹, we have continued the observations through the period of opposition. A reversal in sign of the predominant "polar" effect can now be reported, clearly substantiating a scattering model² as opposed to alternatives such as magnetoreflexion. We have also observed what are believed to be analogous effects, although sometimes with differing signs and other interesting features, in the cases of Mars, Venus, Mercury and the Moon. The data in these cases are illustrative only, but they show that the phenomenon is general, that it can be measured and that it provides new information for planetary astronomy.

All these observations except those on the Moon followed a scheme shown in Fig. 1a. Fractional circular polarizations Q_0 , Q_N , and Q_S were measured in light from the whole planet, the northern hemisphere, and the southern hemisphere respectively. North and south in all cases are heliocentric directions (normal to the plane of the solar system) rather than terrestrial or planet-axis directions. For Q_N and Q_S a large aperture stop was used at the telescope focal plane, two to three times the diameter of the planet image; the stop edge served to bisect the planet approximately as shown. In the earlier Jupiter measurements¹, Q_N and Q_S were measured instead with a small stop, about half the diameter of the image of the planet and centred inside the planet disk near one or the other of the poles. The new choice gave very similar values (as checked on Jupiter) and reduced the problem of guiding, as the principal drift was in right ascension. It should be approximately true that $Q_0 = \frac{1}{2} (Q_N + Q_S)$; in the data reported this is not always so, notably in the case of Mercury, because of telescope guiding errors. Contamination by sky

effects was always checked and was possibly significant only in the case of Mercury. Spurious signals due to the indirect effect of linear polarization were cancelled out routinely, as in previous work, by taking the algebraic average of readings made with the polarimeter at relative orientations 0° and 90° about the telescope axis. The bulk of the measurements were made on one of the two 24 inch telescopes at Mauna Kea Observatory; on Jupiter, Venus, and Mars, comparison measurements were made with the 88 inch telescope, with entirely consistent results.

With the exception of Mars we have confined virtually all the measurements to a pass band about $500 \text{ \AA}$ wide (half-transmission points) centred near $6800 \text{ \AA}$, for reasons indicated earlier¹. Actually the spectral coverage can be readily extended.

Jupiter

The crossover of Q_N and Q_S (hemispheric polarizations) through zero at opposition of Jupiter is apparent in Fig. 2. The abscissa here is the phase angle ϕ between the Sun-planet and Earth-planet directions. (This angle was denoted ψ in the earlier papers^{1,2}; we have changed notation to conform to that of Pospergelis³, whose laboratory work will be discussed here.) Before opposition, or when the planet is a "morning" object, ϕ is negative. We observe that Q_N goes almost linearly through zero at $\phi=0^\circ$; this is the behaviour expected over the small range of ϕ represented here. A sur-

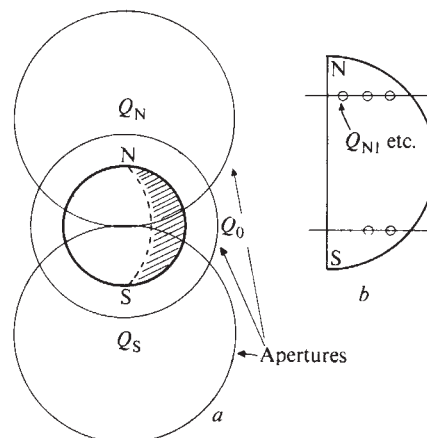


Fig. 1 Observing schemes for measuring the circular polarization of the whole planet Q_0 , and hemispheric values. The lighter circles in a are telescope aperture stops. a, Planet; b, Moon.

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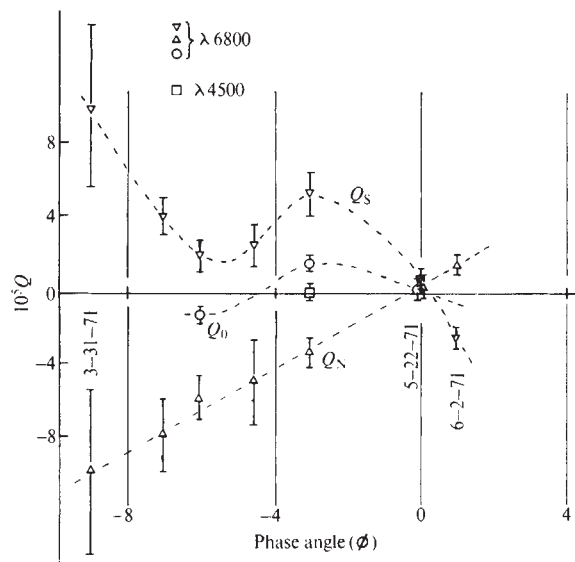


Fig. 2 Fractional circular polarization of Jupiter during the period of opposition, May 23, 1971. Observation on June 15, at $\phi = +4.5^\circ$, after this figure was drawn, gave $Q_0 = +1 \times 10^{-5}$, $Q_N = +5 \times 10^{-5}$, and $Q_S = -6 \times 10^{-5}$, all at $\lambda = 6800 \text{ \AA}$.

prising peculiarity, however, is seen in Q_S —a kind of S-shaped undulation, superimposed on the overall crossover through zero. This undulation is also reflected in the polarization, Q_0 , of the whole planet. The cause of this fluctuation cannot be a general north-south asymmetry with respect to absorption; that would lead merely to a difference in slopes between the Q_S and Q_N curves. One question is whether the Q_S anomaly can somehow be related to the presence of the Red Spot in the southern hemisphere. We did not monitor Q_S and Q_N over a complete cycle of the planet's rotation cycle, at each orbital phase ϕ . But in one case, the observations of May 10–11 ($\phi \approx -3^\circ$), we measured the quantity Q_0 periodically over a five hour period, during which the Red Spot traversed the front of the planet and disappeared behind the west limb. Q_0 did not change by more than the measurement uncertainty of about $\pm 20\%$. The cause might instead be related to the orientation of Jupiter's spin axis, which is inclined about 3° from the normal to the orbit plane and which, during the period of this opposition, was such that the south pole was tilted toward us by the maximum amount.

In the observations of May 10–11 when Q_0 showed a maximum positive value, we also measured this quantity with a broad blue filter, having a pass band of $1000 \text{ \AA}$ centred at about $4500 \text{ \AA}$. The null result $(0.0 \pm 0.5) \times 10^{-5}$ is consistent with the notion that ellipticity requires absorption. Studies in the near infrared, where there are strong methane bands, will be expected to show large Q values.

No further results were obtained on the interesting localized effects noted earlier in the area of the Red Spot¹. These must await construction of an improved arrangement for guiding the telescope which will facilitate signal-averaging on small areas of the image.

Venus and Mercury

Having a dense atmosphere as does Jupiter, Venus showed the polar scattering effect, as expected, with the same signs of Q_N and Q_S as Jupiter for the same sign of ϕ . (In this instance Venus was a morning star, with negative ϕ as with Jupiter before opposition.) What one might call the elliptical scattering power Q/ϕ (or $\partial Q/\partial \phi$) is notably smaller (Table 1) than for Jupiter, at the same measuring wavelength in the deep red.

The single result on Mercury showed the same sign of the polar effect as for Jupiter ($\phi < 0$) and Venus, but with a surprisingly large asymmetry that is a large Q_0 , as shown in Table 1. As with observations of other types on Mercury⁴, this measurement was a precarious one which had to be accomplished within a period of less than 30 min while the planet was high enough to be free of horizon effects, yet before sunrise. Seeing conditions were just adequate to permit us to discern the halfmoon-like image, and resolution of the planet into north-south halves (Fig. 1a) was done with only fair accuracy. We were aware that an elliptically polarized morning sky could in this case be a spurious cause of the asymmetry, adding, for example, to Q_S and acting to cancel Q_N . A brief test on adjacent sky tended to rule this out, and we believe the asymmetry is real. Whether or not that is so, the polar difference $Q_N - Q_S$ should be independent of sky effects and clearly has the same sign as in the case of Venus. We will comment later on the significance of this sign.

Mars

A striking wavelength-dependent asymmetry was observed in the case of Mars, shown in Fig. 3. In the deep red, the circular polarization seems to approach the ideal polar asymmetry, $Q_N = -Q_S$, although with signs opposite to those of Jupiter and Venus. In the blue, however, the polarization on both poles was overwhelmingly negative. On the basis of the visual appearance of the planet on the dates of the observations, some kind of asymmetry could indeed have been anticipated. The Martian season corresponded to spring in the southern hemisphere, the south pole being tilted toward the Sun with the south polar cap a very dominant feature. A consistent, although quite tentative, explanation for the behaviour of Q_N and Q_S is as follows. At long wavelengths the atmosphere is unimportant, and the scattering is characteristic of a solid surface. That a solid surface can in fact give a polar effect of the sign observed, for the orbital phase angle obtaining here (-40°), will be indicated later. In the blue, on the other hand, there is appreciable atmospheric absorption, the Q values tending to switch over to the "Jupiter" signs. But the albedo of the south polar cap is so large that most of the light from the south comes from solid-surface reflexion, washing out the oppositely polarized flux associated with atmospheric scattering in that region. In the blue, therefore, the atmospheric sign only achieves dominance in the north, where the mean albedo is low.

Uranus and Neptune

Data for Q_0 for Uranus and Neptune are included in Table 1, but are to be taken merely as upper limits. They represent

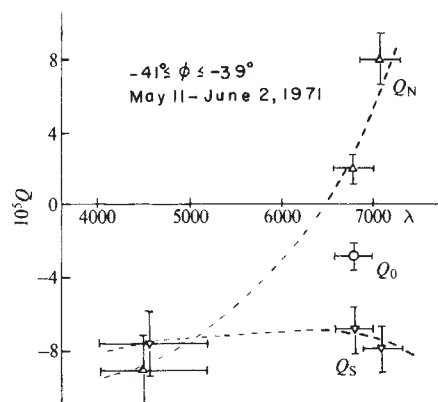


Fig. 3 Crude wavelength dependence of the circular polarization values on Mars. The horizontal error bars are pass bands of the filters used, weighted by the photomultiplier response.

Table 1 Fractional Circular Polarizations in Units of 10^{-5}

	Dates (1971)	Mean ϕ	Mean λ	Q_0	Q_N	Q_S
Venus	May 20, 23; June 1	-37°	6,800	—	$-(7 \pm 2)$	$+(5 \pm 1)$
Mercury	June 2	-67°	6,800	$+(12 \pm 4)$	$-(2 \pm 2)$	$+(18 \pm 6)$
Moon	May 31	$+90^\circ$	6,800	—	$+(4 \pm 1)$	$-(3 \pm 1)$
Uranus	May 23	$+2^\circ$	5,000	(0 ± 1)	—	—
Neptune	June 1	0°	5,000	$-(20 \pm 40)$	—	—

signal averaging over one hour on the 88 inch telescope (Uranus), and about three hours averaging on a 24 inch telescope (Neptune). As far as the polar scattering effect is concerned, if these planets are reasonably symmetric no finite values of Q_0 are expected. It seems feasible to resolve Uranus into north and south halves in a future measurement.

The Moon

The observing scheme in Fig. 1b was used to measure Q values on the Moon. Here it was not convenient with the existing polarimeter and telescope optics to make hemisphere measurements. We used an aperture diameter of about 1 arc minute, and selected a sequence of three mirror-image pairs of regions located at complementary northern and southern lunar latitudes of very roughly $\pm 45^\circ$, as shown in the sketch. The regions were selected to be as far as possible free of severe relief features. Regions N1 and N3 (and southern counterparts) were near the terminator and the limb respectively, with N2 intermediate. In either hemisphere the Q values for locations 1, 2, and 3 were within a factor of three of each other and had the same sign. The two averages Q_N and Q_S of each of these sets of three values are entered in Table 1; these may reasonably be compared with the hemispheric averages measured on the planets. The phase here was first quarter (half Moon waxing), corresponding to $\phi = +90^\circ$. The polar values are seen to have the same signs as for Jupiter at positive ϕ .

Models for the Polarization

The rudimentary model for elliptically polarized scattering at a gaseous surface² is consistent with the signs of the polar effect on Jupiter and Venus. What, however, happens with solid surfaces, as on the Moon? Although no theoretical treatment has been given, we may appeal to an extensive laboratory study by Pospergelis³ of the ellipticity of initially unpolarized light as scattered from a variety of rock and mineral surfaces. For comparison we must point out that our Q equals $-S$ in his notation, where S is the conventional fourth Stokes parameter. His analysis involves certain angles—the phase ϕ (which has the same meaning as in this article), and the angles γ and ψ giving the orientation of the plane scattering surface. In our context we interpret γ and ψ as the latitude and longitude respectively of a local scattering region on a sphere. (The region on the sphere nearest the observer has $\psi = 0^\circ$.) His results for the circular polarization show, as well as the quite general antisymmetries $Q(-\phi) = -Q(\phi)$ and $Q(-\gamma) = -Q(\gamma)$, sign change on variation of ϕ , not dictated by symmetry considerations. The situation is complicated by the behaviour with respect to ψ ; but for scattering from the entire north or south half of the visible surface of a planet at phase ϕ , there is a mean ψ roughly equal to $\phi/2$. For $\psi = \phi/2$, Pospergelis's data for all surfaces investigated (excluding unusual materials with optical activity) indicate that for all ϕ between 0° and a critical phase ϕ_c the hemispheric values Q_N and Q_S should have opposite signs from the gas-layer case; $Q(\phi_c)$ is zero, and for $\phi > \phi_c$ the signs agree with those of the gas case. Evidently ϕ_c depends on the nature of the surface, but the typical value is around 70° . (Another critical value $\phi \sim 40^\circ$ is mentioned by Pospergelis³ but that

is for $\phi = 0^\circ$. We have deduced $Q(\phi)$ for the case $\phi = \phi/2$ from families of curves given for a typical surface.) The gas-layer model² does not have any such critical angle, $Q(\phi)$ having one sign over the interval $0^\circ < \phi < 180^\circ$.

The mechanism for elliptically polarized scattering from condensed matter is different from the gas-layer case. We are considering a model based on double specular reflexions from the facets of crystal grains exposed on a surface. (Equivalently this could be a layer of aerosol particles large compared to a wavelength.) The first reflexion linearly polarizes the ray; the second, from an adjacent grain, elliptically polarizes it by virtue of the material being slightly absorbing. The critical angle ϕ_c is perhaps related to Brewster's angle for the substance.

The signs of Q_N and Q_S on Mars in the deep red (Fig. 3), at $\phi = -40^\circ$, are consistent with the conclusions for solid surfaces, for it is fairly clear that the magnitude of ϕ is less than ϕ_c . The data for the Moon, at $\phi = +90^\circ$, are similarly consistent as here we seem to have $\phi > \phi_c$. With Mercury the situation is less sure, and little more can be said until investigations at other phases are carried out.

One difference between the data given here and the laboratory results of Pospergelis is in the general magnitudes. His values are of the order 0.1% to 1%, whereas ours are between 0.001% and 0.01%. As noticed empirically³, the elliptical polarizing power is drastically reduced in a powdered sample as compared to polycrystalline rock. On the face of it this implies that Mars, the Moon and presumably Mercury are largely covered with dust or other fine-textured material, and of course the nature of lunar dust is now well known. The suppression of the polarization is probably because the double reflexion mechanism gives way to multiple reflexions, that is, a ray is trapped in surface cavities before scattering out, randomizing the polarization; or perhaps the particle size becomes smaller than a wavelength and Rayleigh scattering occurs, which cannot produce elliptical polarization by the mechanism described.

What meaning does the polar scattering effect have for astronomy? Given a better knowledge of the origin of the effect, it can give information on planetary surfaces. Even without such knowledge the symmetry aspects alone are of immediate use. A dramatic application should be in the study of asteroids. Here we refer to measurement of Q_0 —the integrated effect for the whole body—which is nonvanishing for an asymmetric figure. From Q_0 and its time dependence one can deduce facts about the shape and orientation of the body, complementary to the information furnished by linear polarization and the light curve. Finally, because the effect specifically requires absorption, a spectral curve $Q(\lambda)$ might reveal absorption bands difficult to see otherwise; confusion with absorption bands in the Earth's atmosphere would be almost impossible.

Recognizing the polar scattering phenomenon is necessary before we can search for other causes of circular polarization in light from the planets, such as magnetoreflexion or circular dichroism. The latter is important in the detection of biological molecules. For example, the possibility of Martian plant material having optically active scattering properties comparable to those of green leaves³ can be tested by searching for the circular dichroism band at 7000 Å. As the circular-dichroic ellipticity neither vanishes nor changes sign at $\phi = 0^\circ$,

such an effect would stand out clearly at opposition. (It happens that our two filter points at 6800 Å and 7100 Å in Fig. 3 approximately straddle that particular band, although they were not selected with such an investigation in mind. The rapid change in Q values between the two wavelengths is likely to be related to the transition from solid-surface to atmospheric scattering, as indicated earlier.) In forthcoming observations a monochromator will be used to study $Q(\lambda)$ with a resolution of about 20 Å on Mars and other planets.

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The Evolution of the North-East Atlantic

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Magnetic data taken over a sixteen year period have been analysed and information on the evolution of the North-East Atlantic has been deduced.

It is well known that the evolution of ocean basins can be worked out in considerable detail from magnetic lineations observed at sea level. This technique has already been applied to the Pacific, Indian and South Atlantic oceans, whose evolution is now known in some detail. Very little work has been carried out so far on the North Atlantic, probably because the slow rate of separation of Eurasia and North America has

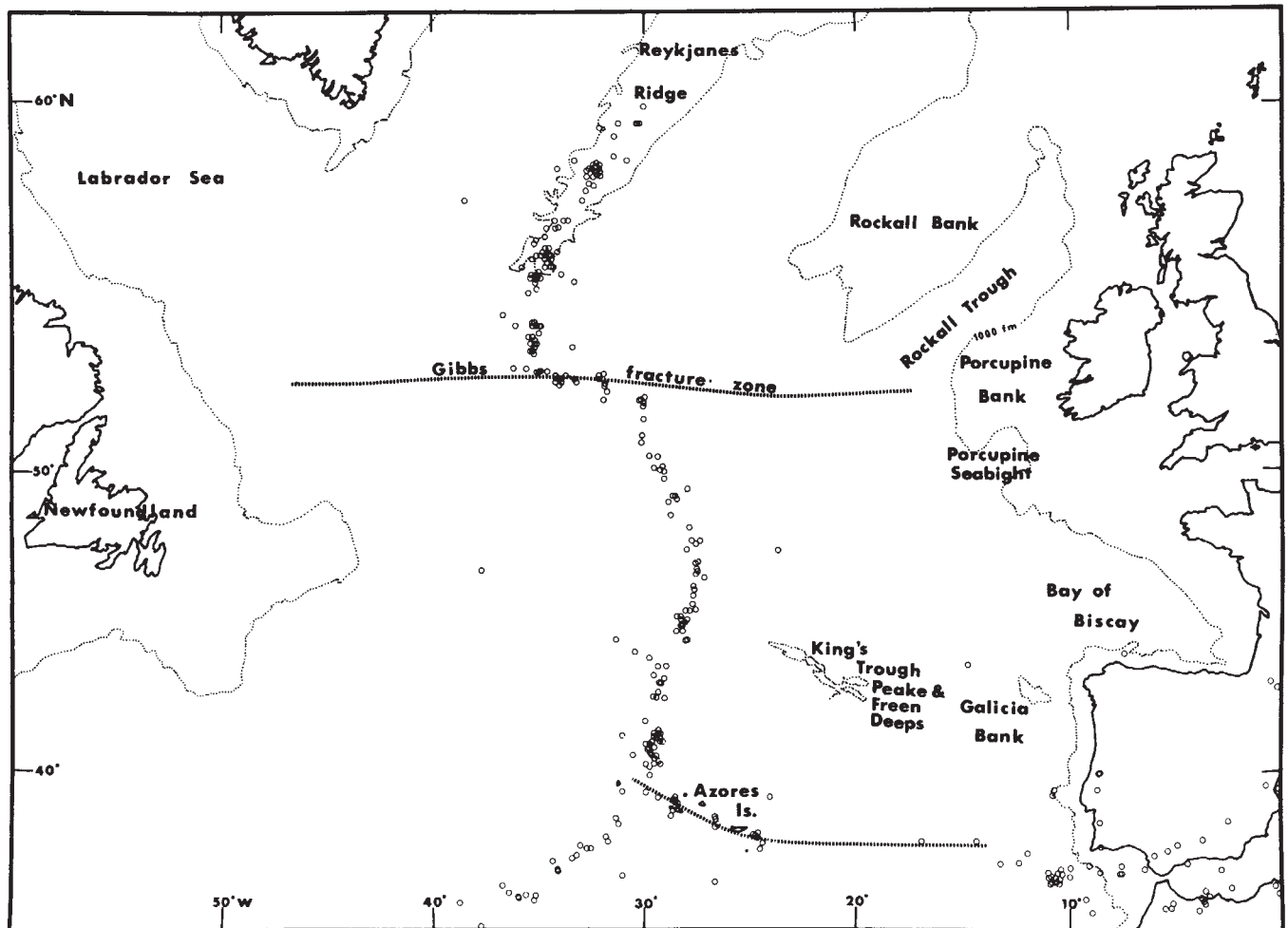


Fig. 1 Location map of the North Atlantic, with USCGS earthquake epicentres.

caused the anomalies to be harder to identify. This is partly because the details of short wavelength anomalies cannot be resolved from the sea surface and also because the seafloor spreading lineations are confused by anomalies from topographic effects and serpentinite intrusions (F. Aumento, personal communication).

Most of our data (Fig. 2) lie within the region bounded by the Azores-Gibraltar Ridge, the Mid-Atlantic Ridge, the Gibbs fracture zone and the continental margin. It is this area with which this article is primarily concerned. It is, however, particularly difficult to apply plate tectonics to a restricted region and our interpretation also depends upon anomalies identified by Pitman and Talwani in the North-West Atlantic¹.

The principal features of the seafloor in this region are the Azores-Gibraltar Ridge, which is seismically active, Kings Trough and Peake and Freen Deeps², the Gibbs fracture zone at 53° N and the present ridge crest. The continental margin from southern Spain to 53° N contains four major indentations. The largest of these is the Bay of Biscay. The other three are Porcupine Seabight³ and Rockall Trough (Fig. 1) and the southern end of Hatton-Rockall Basin⁴.

The other major feature on the continental margin is an elongated sediment-filled trough, parallel to the north coast of Spain⁵, and characterized by a large negative gravity anomaly. These features must have been formed since the separation of Europe and North America and the evolution of the North Atlantic, recorded by the magnetic lineations, should help to understand their origin.

Magnetic Anomalies

Magnetic measurements have been made in the North-East Atlantic by the Department of Geodesy and Geophysics, Cambridge, since 1956, and wherever possible these original records

were used. Other data collected by the British and Dutch Navies⁶ and Bedford Institute, Canada (C. A. Williams and D. M. Porteous, account in preparation, and D. I. Ross, Bedford Institute Geophysics report, 1967), were also used, though not in the form of the original records. These magnetic data were digitized and the International Geomagnetic Reference Field⁷ removed. The resulting anomaly profiles were plotted at right angles to the ships' tracks on 1 : 1 m scale charts at a scale of 200 γ to 1 inch. The strike of the lineations obtained from these charts was then used and the ship's tracks projected perpendicular to it. The anomalies were identified with the help of synthetic profiles at exactly the same scale and strike. A more detailed description of this procedure can be found elsewhere⁸.

The profiles projected at right angles to the strike of the lineations are shown in Figs. 3 and 4 and the lineations themselves in Fig. 6. The synthetic profiles were obtained from the reversal time scale of Heirtzler *et al.*⁹ with McKenzie and Sclater's modification of the time scale beyond anomaly 30. A depth to magnetic basement of 3.3 km was assumed from the ridge axis to anomaly 5 and of 5 km beyond anomaly 5. The reduced amplitude of the older part of the synthetic profile is probably due to a palaeolatitude effect since this part of the ridge was then closer to the magnetic equator. Since the lineations change trend in this region the strike of the line perpendicular to them is not constant.

The profiles in Figs. 3 and 4 show that all the anomalies from 1 to 32 are present in this part of the North Atlantic. This conclusion differs from that of Talwani, Pitman and Heirtzler¹¹ and Pitman, Talwani and Heirtzler¹ who consider that there was a pause or extreme slowing down in spreading between 38 and 9 m.y. ago (anomalies 13-5). Their identification of anomalies at the beginning and end of the sequence agrees with that in Figs. 3 and 4. The main difference between



Fig. 2 Ships' tracks along which magnetic data are available. Areas of detailed survey are outlined.

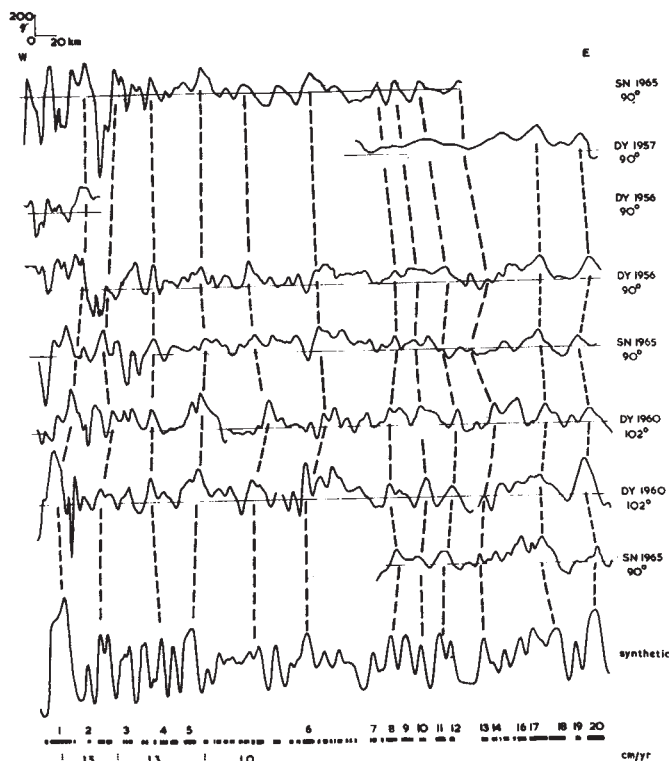


Fig. 3 Projected anomaly profiles from anomaly 1 at the Ridge Crest to anomaly 32. The cruise identification and the directions along which the tracks are projected are shown. The angle is not constant since there is a bend in the lineations in this region. The synthetic profile was calculated after Heirtzler *et al.*⁹ and the positively magnetized blocks are numbered according to the convention of Pitman and Heirtzler¹⁰. The complete sequence of anomalies can be identified. The synthetic profile is calculated at 45° N, except beyond anomaly 24 where a palaeolatitude of 25° N was used. Cruise identifications: Dy, RRS Discovery; Sn, H. Neth, M. S. Snellius 1965 project NAVADO lines J to N; H, CSS Hudson; JM, RRS John Murray; B, CSS Baffin.

these two interpretations depends upon which anomaly is identified as anomaly 13. Fig. 5 shows the two interpretations of this crucial section together with the projected profiles on which the anomalies are most easily recognized. Pitman and Talwani believe 13 to be an anomaly closer to the ridge crest than the one we have identified.

The reversal time scale is not yet known beyond anomaly 32 (79 m.y.). Immediately preceding this there is thought to have been a period of approximately 50 m.y. with no reversals¹². Seafloor formed during this period is known as the quiet zone in the Pacific. The seafloor east of anomaly 32, where no lineations are observed, was probably formed at this time. It is, however, important to point out that there are other explanations for the absence of magnetic anomalies since lineations are easily destroyed.

On the eastern side of the mid Atlantic Ridge there are two regions where anomalies are found which are not part of the numbered sequence formed since the Upper Cretaceous (Fig. 6). The first region is the Bay of Biscay where a pattern of magnetic lineations fanning from the South East corner of Biscay have been mapped by Matthews and Williams¹³. The strike of these lineations fanning from the south-east corner of Biscay have northern Biscay they appear to be truncated by part of the quiet zone to the east of anomaly 32, suggesting that the Biscay sequence is older than anomaly 32¹⁴. As there is evidence of reversals here, the seafloor appears to be more than 50 m.y. older than anomaly 32, that is older than 125 m.y. which is lower Cretaceous.

The second region where older anomalies occur is off South-West Spain, where a group of lineations have approximately north-south strikes and fan out in a similar way to those of

Biscay (Fig. 6). One of these anomalies is anomaly J and has also been identified on the western side of the Ridge¹.

Evolution of the North-East Atlantic

The south of the North Atlantic began to open in the Late Trias with the separation of North America and Africa¹. In pre-upper Cretaceous times the spreading ridge extended northwards into Biscay. The fanning of the Biscay anomalies and also those off South-West Spain suggests that both sets were formed synchronously accompanied by the anticlockwise

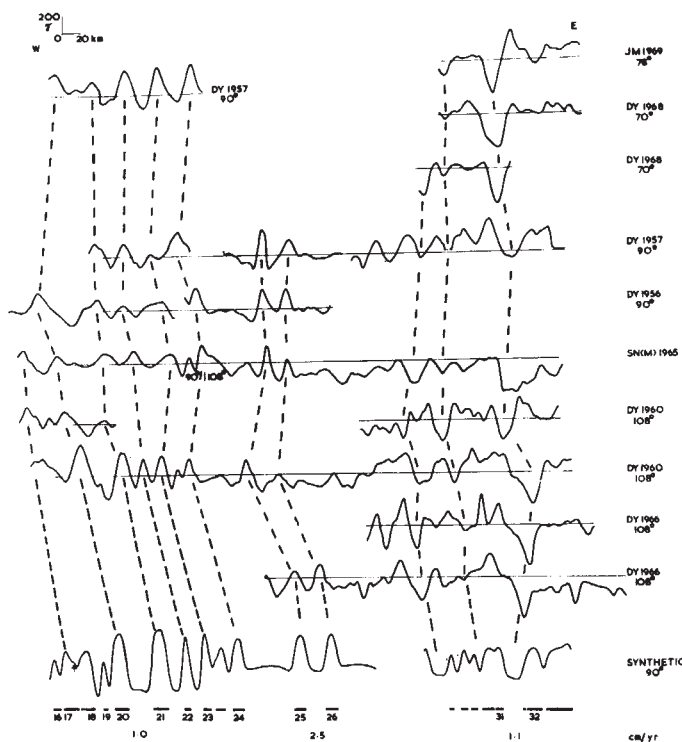


Fig. 4 See caption to Fig. 3.

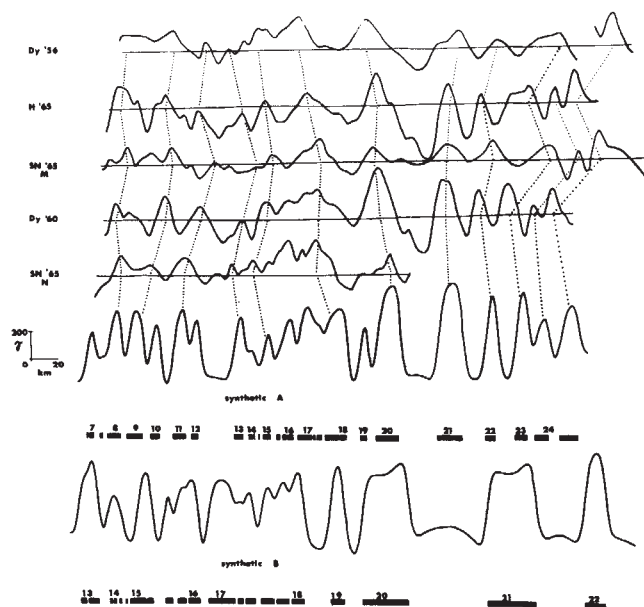


Fig. 5 The identification of anomaly 13. A and B are synthetic profiles. A according to this interpretation with a spreading rate of 1 cm yr⁻¹ and B according to Pitman and Talwani with a spreading rate of 2 cm yr⁻¹ for comparison. Anomalies 24 to 20 are easily recognized and the decrease in spreading rate can be seen across anomaly 24 and in Fig. 4. Cruise identifications as for Fig. 3.

motion of Iberia. If this suggestion is correct, then one of the Biscay anomalies is anomaly J. It seems likely that the Bay of Biscay was formed by the rotation of Spain in the Upper Jurassic or Lower Cretaceous.

By the time of anomaly 32, Biscay had ceased to be active and the spreading ridge extended north-westward to the north of Biscay (Fig. 7). The motion at the northern end was probably taken up by the Gibbs Fracture Zone. By the time of anomaly 31 the ridge extended beyond the fracture zone into the Labrador Sea¹⁵. The northern part of anomaly 32 is sub-parallel to

the continental shelf edge and crosses the mouth of Porcupine seabight. This feature was therefore probably formed before anomaly 32 during the early phases of separation of Europe and North America. The seabight, Rockall Trough and Hatton-Rockall basin are likely to have been formed by the ridge attempting to extend by breaking through the plate to the north. This does not necessarily imply that these are floored by oceanic crust, however, as they may have been produced solely by extension of continental crust.

Anomaly 32 is less easy to identify than the negative anomaly

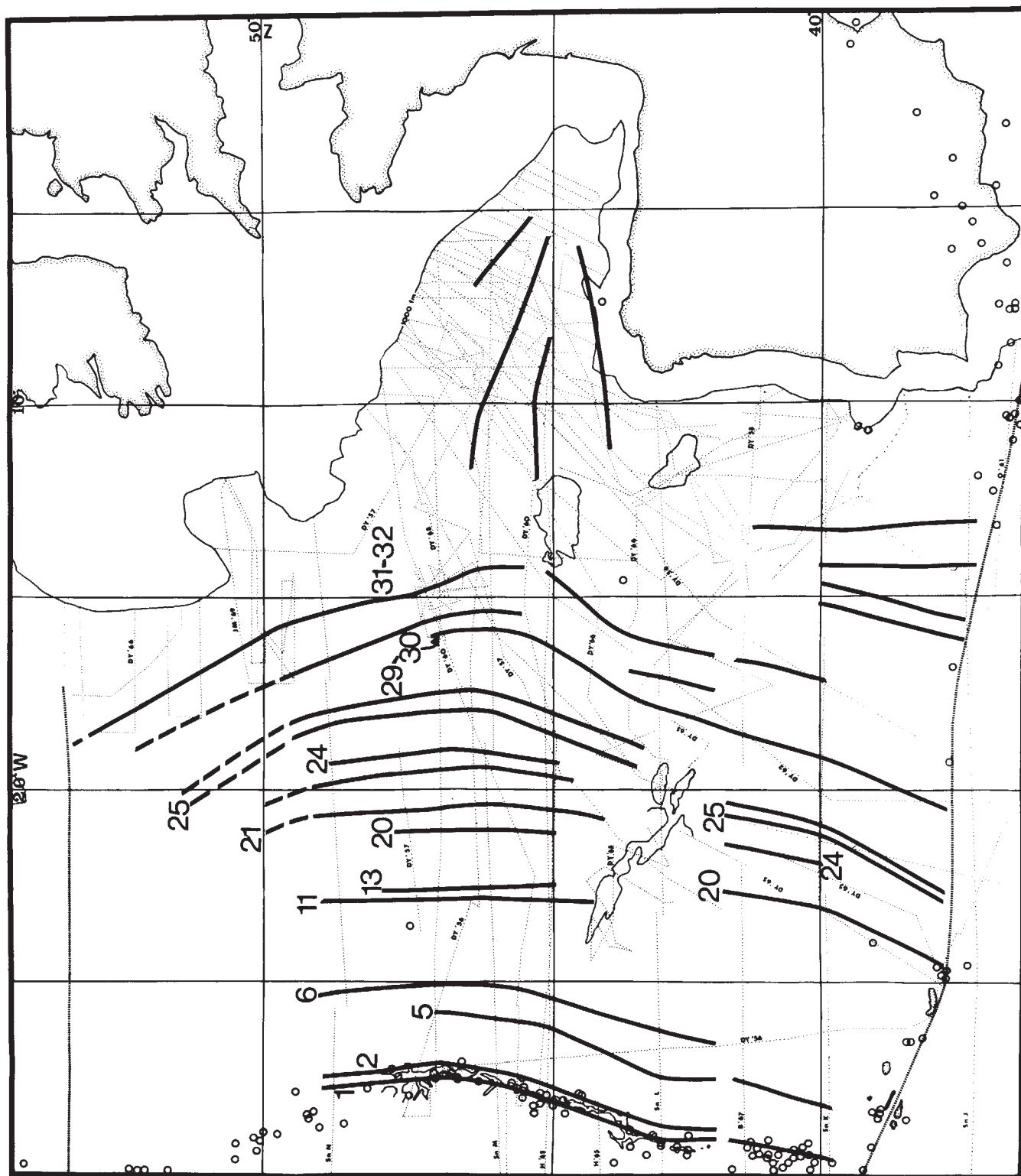


Fig. 6 Magnetic lineations. Only the most prominent ones are shown; the ships' tracks are dotted and indicate the control on the lineations. Cruise identifications as for Fig. 3.

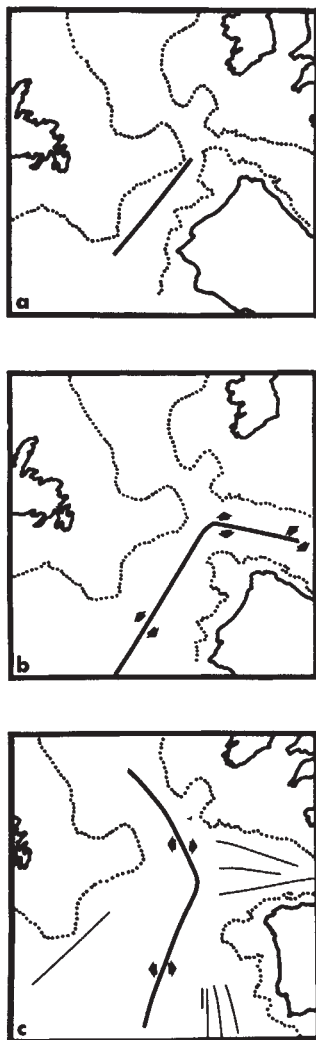


Fig. 7 Diagrammatic reconstruction of early opening of Biscay and the North Atlantic. The thick line represents the Median valley. *a*, Early Mesozoic extension between Iberia and Newfoundland; *b*, extension of the spreading centre into Biscay; *c*, extension north of Biscay about 80 m.y. BP (anomaly 32).

between 31 and 32 (Figs. 4 and 6), which forms a striking feature on most profiles. The 31–32 lineations have a north-easterly trend south of Biscay, which changes off the mouth of Biscay and becomes north-westerly. The adjacent younger lineations also show this changing trend. Similar anomalies have been found in the North-East Pacific, where the lineations forming the Great Magnetic Bight have two straight segments meeting at an angle. Unlike the North Pacific anomalies, however, the eastern north Atlantic lineations young in the concave direction. They are therefore difficult to explain by the evolution of a triple junction similar to the Great Magnetic Bight. Moreover, Pitman *et al.*¹ have identified similar shaped lineations on the opposite side of the Atlantic. If the bend in the lineations on both sides of the ridge is fitted together, the motion required to do so is essentially parallel to the Gibbs fracture zone, and therefore the ridge must have been spreading obliquely to the lineations at this time.

This curious geometry is not easily explained, because if a ridge spreads symmetrically it cannot change its shape. Two possible mechanisms which would account for the observations are that spreading was asymmetric or that the ridge jumped, forming several small offsets. These two possibilities and their consequence are illustrated in Fig. 8. The present density of ship tracks is not sufficient to choose between the two mech-

anisms, and if there are indeed small fracture zones present in this area the strike of the lineations in Fig. 6 will not be correct. It should be straightforward to do this by a detailed survey, especially if satellite navigation is available. Anomalies west of 31 are presumably 29 to 27, but cannot be numbered with any certainty. Anomalies 25 and 26 are, however, easily recognized on most tracks. Though these lineations are sub-parallel to anomaly 31, careful measurement of their strikes shows that the ridge has become straighter with time. This process continued until anomaly 24, and after anomaly 23 the lineations are straighter on both sides of the Atlantic. These events are closely related in time to the extension of the ridge to the north-east to form a triple junction south of Greenland¹⁶ at anomaly 24 time. There is also a slight change in trend of the lineations in Fig. 6 at anomaly 18 time, which is the time at which the spreading ceased in the Labrador Sea.

The approximate spreading half-rates were 1.1 cm yr^{-1} at anomaly 32 time, between anomalies 26 to 24 spreading increased to 2.5 cm yr^{-1} and slowed to 1 cm yr^{-1} at anomaly 23. Spreading continued at 1 cm yr^{-1} until anomaly 6 when it increased to 1.3 cm yr^{-1} . At anomaly 5 it increased again to the present rate of 1.5 cm yr^{-1} .

Bacon and Gray¹⁷ have suggested that Spain moved eastward relative to Europe after the Bay of Biscay opened. The magnetic anomalies in Fig. 6 do not permit a large strike-slip motion after anomaly 32 was formed, and therefore any such motion must have occurred before the Upper Cretaceous. The most likely explanation for the sedimentary trough north of Spain is that it was produced by a small amount of crustal consumption along this boundary, perhaps during the Eocene when the Pyrenees were folded.

Fig. 6 also shows that there is no major horizontal displacement across Kings Trough and Peake and Freen Deep. Therefore these features are not the relics of a large transform fault. They cut anomalies from 24 to the first part of 6 and may well be connected with the elimination of the bend in the ridge axis at anomaly 24 time and subsequent small changes in ridge orientation which took place during the period of anomalies 24 to 6.

This study of the magnetic lineations shows that the formation of the North-East Atlantic began with the separation of Spain from North America and Europe in probably the Upper Jurassic and early Cretaceous. The ridge extended northwards as far as the Gibbs fracture zone at 53°N by the Upper Cretaceous and beyond this into the Labrador Sea by anomaly 31 time. Most of the complications of the European continental margin and surrounding seafloor appear to be the result of this intermittent northward extension of the mid-Atlantic Ridge into Biscay, possibly beginning to extend into Porcupine

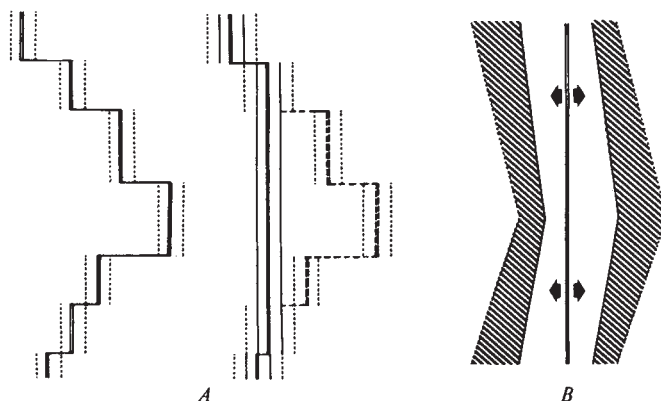


Fig. 8 Two possible mechanisms to account for the shape of the observed lineations. *A*, The ridge straightens by reducing the number of offsets. *B*, Spreading is asymmetric across certain segments of the ridge. This has not been conclusively shown to occur.

seabight, Rockall Trough and Hatton-Rockall basin. Sometimes these extensions were long term and sometimes very temporary. This behaviour is strikingly similar to that of the ridge in the Indian Ocean⁸.

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Establishment of Symbiosis between *Rhizobium* and Plant Cells *in vitro*

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A symbiotic nitrogen-fixing relationship has been established in culture between soybean cells and the bacterium *Rhizobium*.

SYMBIOTIC fixation of atmospheric nitrogen by leguminous plants is perhaps the most quantitatively important agency by which this element is incorporated into the biosphere, and is the principal process for maintaining nitrogen fertility in agriculture. The interaction of plants of the family Leguminosae and specific bacteria of the genus *Rhizobium* in this symbiotic relationship is not yet well understood, probably because of the absence of a suitable defined experimental system. Other workers have used isolated plant roots in combination with the appropriate bacteria¹⁻⁴, and, more recently, plant cell and tissue culture⁵. Although stimulation of plant cell differentiation and lignification apparently resulted, no evidence for intercellular bacterial symbiosis could be found.

We have attempted to establish a symbiotic N₂-fixing relationship with plant cells and microorganisms *in vitro*, using the techniques of aseptic plant cell culture in conjunction with the sensitive acetylene-ethylene assay for nitrogenase activity. We consider that such a system can be used further to elucidate the factors which control nodulation processes and N₂-fixing activity *in vivo*.

Soybean Root Cell Cultures

Seeds of soybean (*Glycine max* var. Acme) were surface sterilized by immersion in a commercial hypochlorite solution ('Zonite', Chemway Corp., Wayne, New Jersey) for 15 min, and rinsed with sterile distilled water. Seed was placed on the surface of an agar (1% w/v) solidified medium composed of the mineral salts (MS medium)⁶ supplemented with sucrose, 30 g/l., for germination in the dark at 26° ± 1° C. All media were sterilized by autoclaving at 121° C for 15 min.

When the primary root was approximately 4 cm long, it was excised aseptically, and a 5 mm explant was removed 2.5 cm behind the growing tip. This explant of root origin was placed on the surface of 50 ml. of agar-solidified MS medium supplemented with 15% whole coconut milk (CM, Grand Island Biological Co.) and 2 mg/l. 2,4-dichlorophenoxyacetic acid (2,4-D) contained in a cotton stoppered 250 ml. Erlenmeyer flask. The medium was adjusted to pH 6.0 before autoclaving. The explanted root section was incubated at 26 ± 1° C in the dark.

An actively growing mass of undifferentiated cells (callus) was produced on the root explant in 10-14 days. The callus was then aseptically removed and portions subcultured to fresh MS medium supplemented with 15% CM and 2 mg/l. 2,4-D. In these and subsequent subcultures, we used a liquid medium similar to that described, with 225 ml. of medium in a modified 1,000 ml. flask (Blaessig Glass Co., Rochester, New York)⁷, as well as 50 ml. agar-solidified medium in 250 ml. Erlenmeyer flasks. The liquid cultures were incubated on a klinostat rotating at 1 r.p.m.⁸ in the dark at 26 ± 1° C. An actively

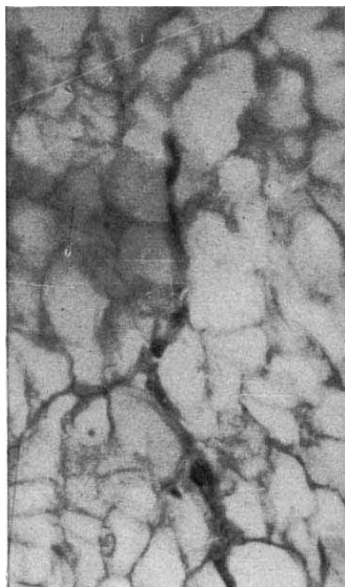


Fig. 1 Paraffin section of a callus culture showing *Rhizobium japonicum* penetration through an infection thread-like structure ($\times 600$).

growing cell and callus population with a cell doubling time of the order of 18 h was established in the liquid environment over a 14–21 day period. Subculture of the liquid system was carried out at 20–30 day intervals to maintain an actively dividing cell population. Cotyledonary tissue was similarly treated to establish cell cultures from this source.

Bacterial Growth

Rhizobium japonicum strains 61A76 (supplied by Dr Joe C. Burton of the Nitragin Co., Milwaukee, Wisconsin) and ATCC 10324 were grown in a liquid culture medium composed of (in g/l): K_2HPO_4 , 1.0; KH_2PO_4 , 1.0; $FeCl_3 \cdot 6H_2O$, 0.005; $MgSO_4 \cdot 7H_2O$, 0.36; $CaSO_4 \cdot 2H_2O$, 0.17; KNO_3 , 0.70; yeast extract, 1.0; mannitol, 3.0. The medium was adjusted to pH 6.4 before autoclaving at $121^\circ C$ for 15 min. A bacterial inoculum was added to 40 ml. portions of the above medium contained in 125 ml. Erlenmeyer flasks and incubated at $30 \pm 1^\circ C$ with constant shaking. Subcultures of the bacterial cultures were carried out at 7 day intervals to maintain vigorous stocks. At regular intervals aliquots of the *R. japonicum* cultures were examined microscopically and checked for nodulating activity by application to seedlings of Acme or Kent variety soybeans maintained in a Sherer-Gillette CEL 255-6 growth chamber under a 16 h photoperiod.

Establishment of a Symbiotic Relationship

A large culture flask from the klinostat containing 225 ml. of MS medium with 15% CM, 2 mg/l. 2,4-D and containing an actively growing root cell and callus population of approximately 15 g fresh weight was inoculated with 0.1 ml. (260×10^6 cells/ml.) of a liquid suspension culture of actively dividing *R. japonicum* and returned to the klinostat for incubation. After 3–7 days of incubation, the flask contents were poured through sterile cheesecloth. Plant cells and callus pieces retained on the cheesecloth (designated stage 1) were washed with 250 ml. of sterile MS medium, transferred to fresh growth media of varying composition, and incubated in the conditions described. Concomitantly, portions of the cell population inoculated with bacteria, as well as control tissue without bacteria, were assayed for nitrogenase activity using the acetylene-ethylene assay procedure⁹. Dry weight determinations were performed after drying the tissue for 48 h at $60^\circ C$.

Cytological Examination of Tissue

Random samples of the soybean root cell cultures from bacteria-inoculated and control flasks were prepared for paraffin sectioning by fixing in formalin : acetic acid : ethanol (5 : 5 : 90 v/v) or in Fleming's osmic acid fixative for 24 h at room temperature. The tissues were then dehydrated through the ethanol series before being embedded in 'Paraplast' (Mh 56° – $57^\circ C$) for sectioning. Sections were cut at 8–10 μm , stained with safranin-fast green or Van Gieson's Nile blue and examined with a Zeiss microscope fitted with phase contrast optics.

At the same time similar cell samples were overlaid with 5% glutaraldehyde in Millonig's phosphate buffer (pH 7.2–7.4) and fixed for 60 min at room temperature. The fixed samples were rinsed for 20 min, post fixed for 60 min at room temperature with 1% OsO_4 , washed with phosphate buffer for 20 min, and dehydrated through an ethanol series up to 100% propylene oxide. The samples were then embedded in 'Epon 812', polymerized at $60^\circ C$ for 24 h, and sectioned at 500 $\text{\AA}$ using a diamond knife. The resulting sections were stained with uranyl acetate followed by Karnowsky's lead and examined in an RCA EMU-3G electron microscope.

Invasion of Cultured Root Cells

Examination of paraffin sections prepared at the time the bacteria-inoculated liquid plant cell cultures were transferred to fresh growth media (stage 1, day 3–7) revealed the presence of structures resembling the infection threads found in *in vivo* nodulating soybean root systems. These "pseudo-infection threads" of the *in vitro* system were observed to penetrate the undifferentiated cell mass for a considerable distance before entering the cell cytoplasm (Fig. 1). *Rhizobia* were observed within the thread-like structures as they traversed the intercellular spaces within the cell mass. Whenever *Rhizobia* entered a plant cell from the terminus of a "pseudo-infection thread", they multiplied to displace the plant cell contents completely with bacteria (Fig. 2). No other morphologically differentiated cells (for example conducting elements) were found in any of the

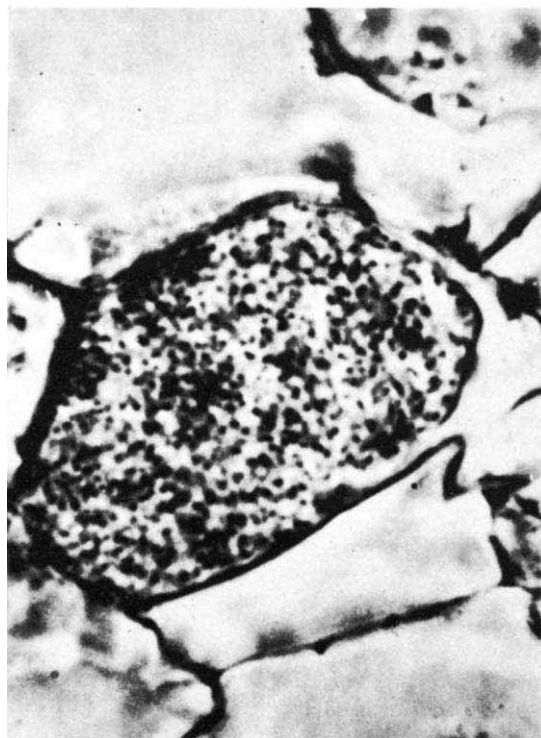


Fig. 2 Callus cell filled with *R. japonicum* after initial penetration.

bacterially populated plant cell masses at this developmental stage.

Continued Development of the Symbiotic System

Following the initial penetration of the plant cells by *Rhizobia* (3–7 days after bacterial inoculation of the cell culture flask), the cell cultures were transferred to fresh media, both liquid and agar-solidified, for development of stage 2. The following media were utilized: (1) MS, (2) MS plus 10–15% whole CM and (3) MS plus 10–15% whole CM and 2 mg/l. 2,4-D. Control cultures of soybean root cells (without bacteria) were transferred to similar media and incubated in the same conditions. All cultures were incubated for 7–20 days after transfer before being examined for bacterial penetration and for nitrogenase activity. Light-grown cultures received approximately 500 foot-candles of light from Duro-Test Optima lamps (Duro-Test Corp., North Bergen, New Jersey).

Fig. 3 shows that the degree of rhizobial invasion after transfer of the cultured soybean root cells is related to the growth factor additions to the MS medium. Most extensive invasion is found after transfer of bacterially populated plant cells to MS medium containing no exogenous growth supplements (Fig. 3a). With the addition of whole CM (Fig. 3b) and the combination of CM and 2,4-D (Fig. 3c) the number of cells inhabited by the *Rhizobia* is markedly reduced. Assays for nitrogenase activity under each of the tested culture conditions (Tables 1 and 2) show this activity to vary directly with the degree of bacterial invasion (Table 1). The tissue without rhizobial involvement shows little acetylene-ethylene reducing activity (Table 1) in these assays^{11,12}. Table 2 shows that although there is wide variation in the response of individual Acme soybean root cell cultures to *R. japonicum* strain 61A76, there is a definite symbiotic relationship capable of fixing N₂ established in liquid systems, as measured by the acetylene-ethylene technique. Attempts to establish an active symbiotic relationship using Kent variety soybean root cells in liquid culture have been less successful. Even with Acme variety soybeans, the use of a different strain of *R. japonicum* (ATCC

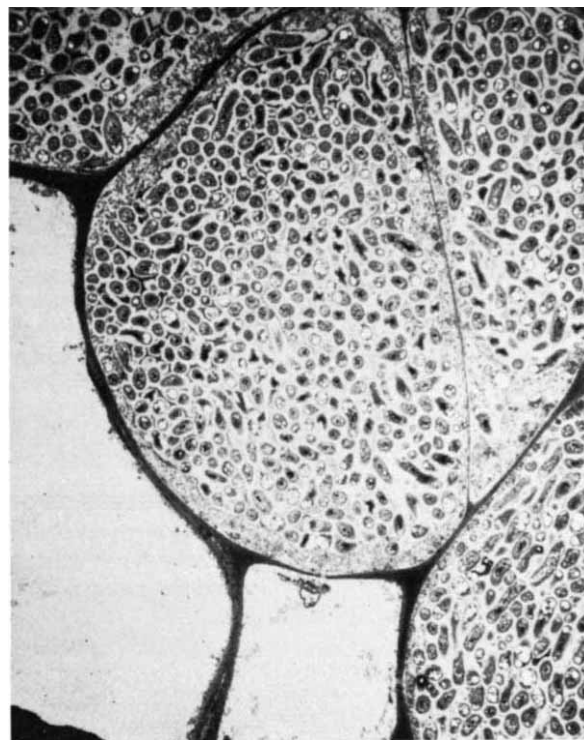


Fig. 4 Electron micrograph of cultured soybean callus infected with *R. japonicum* ($\times 4,160$).

10324) resulted in a lower over-all nitrogenase activity although electron microscopy showed bacterial penetration of the cultured cells. Cotyledonary tissue has shown no propensity to establish a symbiotic association in the conditions of this study.

Table 1 Acetylene to Ethylene Reduction by Soybean Root Cell Cultures inoculated with *Rhizobium japonicum* Strain 61A76

	nmol C ₂ H ₄ /g fresh weight $\times$ 24 h	
	Light	Dark
MS	72.8	318.6
MS + 10% CM	36.6	129.0
MS + 10% CM + 2 p.p.m. 2,4-D	7.7	37.9
Uninoculated control		
MS + 10% CM + 2 p.p.m. 2,4-D	0.4	2.1

The assay was carried out 21 days after initial bacterial inoculation and after transfer to semi-solid agar medium for 14 days. Incubation was under continuous light or dark conditions following initial establishment of symbiosis.

Table 2 Acetylene to Ethylene Reduction by Two Separate Soybean Root Cell Cultures containing *R. japonicum* Strain 61A76 in Symbiotic Relationship

	nmol C ₂ H ₄ /g dry weight $\times$ 24 h	
	1	2
Tissue + 61A76 bacteria	638	88.0
Uninoculated control	307	25.7

Assays were made 3–5 days after addition of the microorganisms to liquid culture.

Electron Microscopic Examination of Symbiotic Cultures

Examination of *Rhizobium*-plant cell cultures with the electron microscope confirmed that *Rhizobia* have entered the cultured plant cells and displaced the normal cytoplasmic components (Fig. 4). In some cases the *Rhizobia* were enclosed

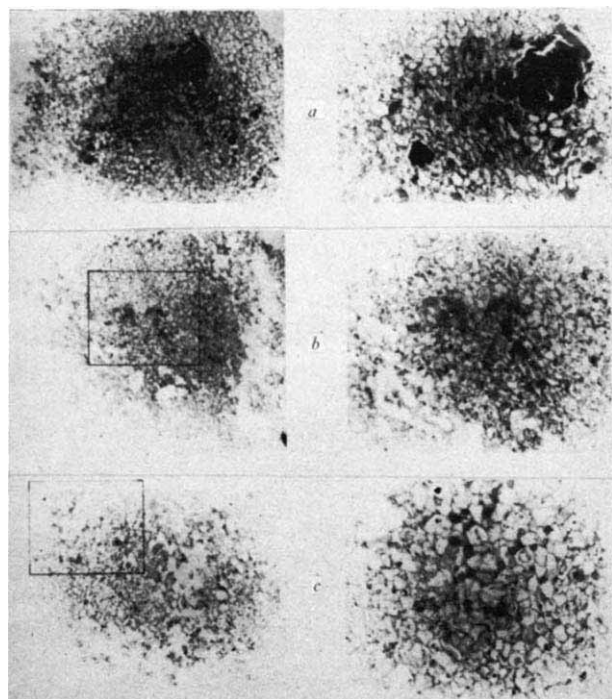


Fig. 3 Sections of callus tissue infected with *R. japonicum* showing the effects of growth supplements on development of the symbiotic association. See text for details of treatments. Left, $\times 350$; right, $\times 700$.

within a vesicle which is morphologically analogous to the vesicles found in nodules obtained from whole soybean root systems (Fig. 5). It was further observed that the *Rhizobia* within the cultured root cells contained an inclusion which was similar in appearance to an inclusion of whole soybean nodule bacterioids designated as a polymer of β -hydroxybutyrate. This inclusion became more prominent with increasing time of incubation of the synthetic symbiotic system (up to 15–20 days after transfer). Microscopic examinations of uninoculated tissue showed that there were no microorganismal contaminants present. The media used for maintenance of the plant cell cultures were also found to be devoid of microorganisms and N_2 -fixing activity; the bacterial inoculum contained only *Rhizobia* and exhibited no N_2 -fixing activity. Thus it is clear that free-living N_2 -fixing bacteria are not responsible for our observations.

Functionality of the Symbiotic Relationship

The functional system we have described provides a new tool for the elucidation of the mechanisms controlling nodulation and subsequent N_2 -fixation in symbiotic associations.

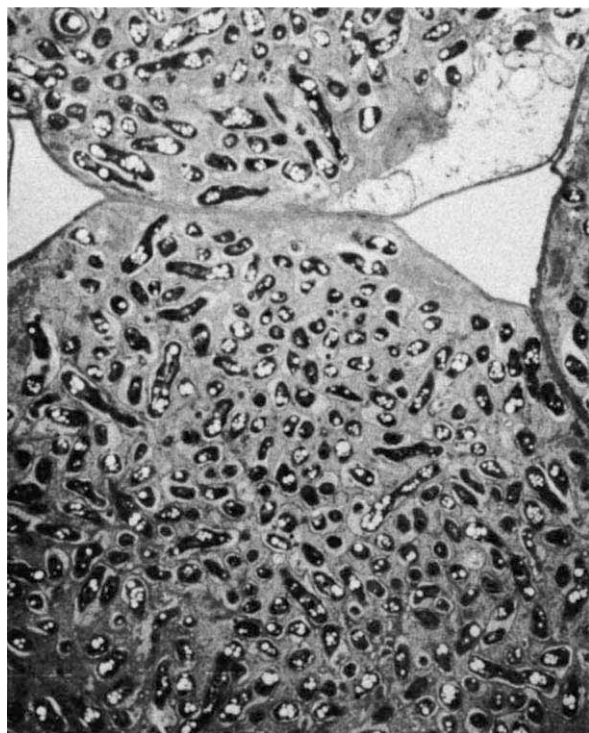


Fig. 5 Electron micrograph of a soybean root nodule infected with *R. japonicum* showing accumulation of β -hydroxybutyrate ($\times 4,160$).

The morphology of symbiosis *in vitro* is analogous to that of intact soybean root nodules^{13,14}. *Rhizobia* within the cytoplasm of the cultured cells, as in root nodules, are at times enclosed within a vesicle. The intracellular bacteria (bacterioids?) are shown to contain an inclusion which has been tentatively identified as poly- β -hydroxybutyrate¹⁵. A structure similar in appearance to the infection thread of nodulating systems *in vivo* has been observed in the early stages of bacterial penetration of the cultured plant cells in the liquid milieu. This apparent mode of rhizobial entry into the cultured cells and the subsequent multiplication of the intracellular microorganisms to displace completely the cell cytoplasm are again analogous to processes which are known to occur *in vivo*.

It has further been found that following initial bacterial penetration of the cultured cells, depletion of exogenous supplies

of plant growth factors (cytokinins and auxins) in the environment leads to increased cellular penetration by the microorganisms and greater nitrogenase induction. This suggests that the symbiosis, once initiated, is self-sufficient and that any exogenous additions tend to suppress its normal development and function. This agrees with the proposed roles of plant growth regulators in the establishment and maintenance of nodulated symbiotic systems *in vivo*^{16,17}. Thus it may be possible to control the process of effective nodulation with factors which alter the levels of endogenous plant growth regulators such as auxins during the critical phases of invasion.

The reduction of acetylene to ethylene as a measure of nitrogenase activity shows that the N_2 -fixing potential of the symbiosis increases with the proportion of cellular invasion by microorganisms, and the addition of plant growth factors depresses nitrogenase activity as well as the development of the symbiotic morphology. It should therefore be possible to increase the overall N_2 -fixing activity of the system by regulating the degree of cell penetration and subsequent nitrogenase induction through chemical means.

Light does not seem to stimulate the symbiosis *in vitro* once it has been established; but cells grown in the light resisted rhizobial challenge, and no working symbiosis could be established in these cultures. It is possible that cells grown in the light develop a natural defence mechanism against invasion by microorganisms *in vitro*, perhaps through the phytochrome system¹⁸.

The acetylene to ethylene reducing activities (and nitrogenase activity by analogy) achieved so far in the *in vitro* symbiotic system are of the order of 1% of those found in soybean nodules. Microscopic examinations of the symbiotic cell masses in culture showed that only 1–10% of the total cell population was infected with intracellular microorganisms. The activity per cell may therefore be comparable with that found in whole root nodules where more than 90% of the cells appear to contain bacteria. It is also pertinent (Fig. 3) that infection occurs only in the subsurface cells of the callus where physiological conditions might be expected to simulate more closely the cortex of intact roots. The initial infection of the tissue culture occurs through a modification of a peripheral cell of the callus mass and not through a root hair cell as in intact roots. This suggests that symbiosis *in vivo* might also be expected to occur through penetration of epidermal cells other than root hairs in suitable conditions.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Search for Isolated Radio Pulses from the Galactic Centre at 151.5 MHz

We have extended our previous work¹ and searched specifically for pulses from the galactic centre (GC), which might be correlated with the events reported by Weber^{2,3}. To discriminate against local interference we looked for pulses received simultaneously by widely spaced receivers; although our results are negative, they allow upper limits to be placed on the radio energy emitted in pulses from the GC.

were no four-fold or five-fold coincidences. The number of coincident events is shown in Table 1 together with the length of time for which such events were possible. The data in Table 1 are restricted to times when the galactic centre was in the beam of the aerials. Data, however, were taken all through the night at some stations, and an analysis of these is shown in Table 2, together with the results of a "random" test in which the times of events were shifted by an arbitrary time relative to each other. From this test we conclude that the real two-fold rates are not significantly greater than the random expectation and we set a three-fold coincident rate, that is the difference between the genuine and the background rate, as 1 in approximately 200 h.

Table 1 Galactic Centre Data

	Two-fold		Three-fold		Four-fold		Five-fold	
	No. of events	Hours	No. of events	Hours	No. of events	Hours	No. of events	Hours
All stations	187	636	4	368	0	209	0	78
CGH	85	512	1	117				
DGM	41	269	0	111				

Four groups have participated, with receivers at Cambridge, Dublin, Glasgow and Harwell, and a receiver in Malta (at 15.5° E, 36° N) has been operated by the Dublin group. The Malta site is 2,450 km from Cambridge, the nearest of the other sites. Each of the stations was equipped with two directional antennas connected as a phase-switched interferometer of spacing 5λ east-west. The antennas were near-vertical ladder arrays of 6 to 8 half-wave dipoles over reflecting screens, providing beams extending about 15° in elevation and directed towards the GC at transit. Our latitudes (51° to 56° N for the UK and Dublin sites) are far from ideal because the GC is visible for only about 6 h and rises to a maximum elevation of 9°. All stations operated at frequencies close to 151 MHz with bandwidths between 0.2 and 1.0 MHz. Cambridge and Glasgow had each a pair of receivers tuned to frequencies 1 MHz apart to measure dispersion and discriminate against interference. The outputs, with integration times between 0.3 and 3.5 s, were recorded on paper charts at speeds between 6 and 24 inch h⁻¹. The observing period was from May 1 to August 16, 1970, that is, when the GC was in transit during the night—and the analysis was restricted to times when the Sun was below the local horizons.

We found many more pulses satisfying the criterion that the amplitude should be at least 3 times peak to peak noise, than in the earlier observations¹. Most of these could be attributed to increased interference arising from the high sensitivity of the antennas at low elevations, and also to the fact that the observations were carried out in the summer when atmospheric disturbances are more prevalent.

As before, the records of the individual sites were scanned for pulses which arrived in "coincidence" (that is, within any 2 min period) at two or more of the five sites. Some two-fold and three-fold coincidences were found, but there

Table 2 Random Test, All Data

	Observed events	Two-fold		Observed events	Three-fold	
		'Expected' events	Hours		'Expected' events	Hours
All stations	242	270	1,114	6	3	586

We studied all the three-fold events in detail and Table 3 shows the date, time and type of each of the six events. All the three-fold events, except for that of July 28, 1970, were made up of pulses observed at three stations while at least one other station had an interference-free chart without any observable pulse. Also three of the events appeared only on one of the two frequency channels at Glasgow and/or Cambridge. The event of July 28 is exceptional because although it appears on both the frequency channels at Cambridge and Glasgow, it is not possible to draw any conclusions from the Dublin or Malta charts. An upper limit of 830 cm⁻³ pc can be placed on the dispersion measure of the source for this event. We conclude therefore that not only were no four-fold coincident radio pulses observed from the galactic centre in 209 h of observation but also that, with the possible exception of the July 28 event, no three-fold events of astronomical origin were observed in 368 h of observation. Weber has divided his events into four classes⁴, roughly dependent on the energy of the event and the characteristic noise near the time of the event (see Table in ref. 4). If this classification is taken as some measure of the gravitational energy in the events, our upper limits on the rate of pulses suggest that there are no radio pulses associated with the two lower energy classes of event, which comprise more than 80% of the total. A consideration

Table 3 The Three-fold Events

Date (1970)	Time (UT) (h) (m)	Class *	Comments
June 11	03 06	CDM $\bar{H}$	Possible interference on D
July 2	20 40	DGH $\bar{M}$	Galactic centre below horizon
July 11	02 14	DGM $\bar{C}\bar{H}$	G pulse on one channel only
July 23	21 29	GHM $\bar{C}$	G pulse on one channel only
July 23	23 04	CGH $\bar{M}$	C and G pulses on one channel only
July 28	20 09	CGH	Both channels on C and G†

* A bar indicates cases in which the traces were clean but there was no evidence for a pulse.

† This event had exceptionally large pulses which were recorded at the three stations at the same times within the limits of error, about 10 s. The mean time, 20 h 09 m 25 s UT, was very close to that of sunset. It was also within a few hours of the outburst⁸⁻¹¹ of Nova Scuti (1970) which happened to be in a sidelobe of our antennas at 20 h 09 m. A further occurrence which could conceivably be related was a transient disturbance in the ionosphere at 19 h 58 m 02 s on the same day, only 11 min earlier. This appeared on a record made at Glasgow of phase fluctuations in a 50 kHz standard frequency transmission from Czechoslovakia. A sudden phase advance of 1 μ s in the received signal was followed by a rapid return to the normal phase within about 2 s. The fast recovery makes this disturbance very unusual; no others like it were found throughout the period of the GC observations.

of the sensitivity of our equipment places an upper limit to the radio energy in our bandwidth of 2.5×10^{-17} J m⁻² per pulse or, on the assumption that the source has a spectrum similar to that of other non-thermal sources, of about 2.5×10^{-14} J m⁻² per pulse in the radio band. This may be compared with Weber's² estimated 10 J m⁻² per pulse of gravitational energy in a bandwidth of 0.017 Hz.

Several factors may complicate such a comparison. (i) The plasma surrounding the source may have a plasma frequency greater than 151.1 MHz, so that the source is completely screened, or the emitted radio spectrum may be quite different from that of "normal" sources. (ii) The time scale of the radio emission may be extended by comparison with that of the gravitational pulses, perhaps because of the emission mechanism or a large dispersion measure. For pulse lengths $T > 1$ s our limiting flux density will be increased by a factor T . The time scale for radio emission from a collapsing object, for example, is likely to be quite different from that of the gravitational pulses. The first of these must originate in the outer regions of the object whereas the second will be associated with the core regions. The recently proposed LeBlanc-Wilson model⁵ of stellar collapse, however, implies a time scale of the order of a few tenths of a second for the emission of most of the radiated energy; (iii) Weber's source or sources may not have been in our beams during the period of observations, because the beam width of his antennas is greater than ours.

Because of these possibilities we emphasize that our findings do not invalidate Weber's results, but simply show that there are no radio pulses from the galactic centre at rates comparable with his rate of detection of gravitational events. When the actual times of gravitational events are published, a detailed comparison with radio observations will be possible.

Partridge⁶ has described an unsuccessful search for micro-wave pulses associated with Weber's events, and Bahcall and Davis⁷ have set an upper limit on the neutrino energy emitted.

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Rapid Optical Variability of the Source PKS 1514-24

THERE are two cases in which radio sources have been identified with optical objects previously known as variable stars: the Seyfert galaxy 3C120 with the star BW Tau (ref. 1) and VRO 42 22 01 with BL Lac (ref. 2). As a systematic search for possible new coincidences, 807 variable stars were selected from the *General Catalog of Variable Stars*³ and its first supplement⁴ using the following criteria: (i) type given as irregular or unknown; (ii) no spectral type given; and (iii) galactic latitude $|b_l| > 10^\circ$. This list was checked for coincidences against *A Master List of Radio Sources*⁵ and the fourth instalment of the Ohio survey⁶. Only two coincidences were found.

The source ON 231 lies close to the star W Com (variable from magnitude 13 to 15.5, type unknown). The position of W Com was measured on the Palomar Sky Survey, and a more accurate radio position was kindly provided by M. Davis. As Table 1 shows, the discrepancy is about two standard errors in both coordinates.

The image of W Com on the Palomar Sky Survey is starlike, but a faint nebulosity seems to extend toward the radio position. A more accurate radio position is needed to ascertain the identification.

The source PKS 1514-24 has been identified by Bolton *et al.*⁷ as an E galaxy. This object had been discovered previously as

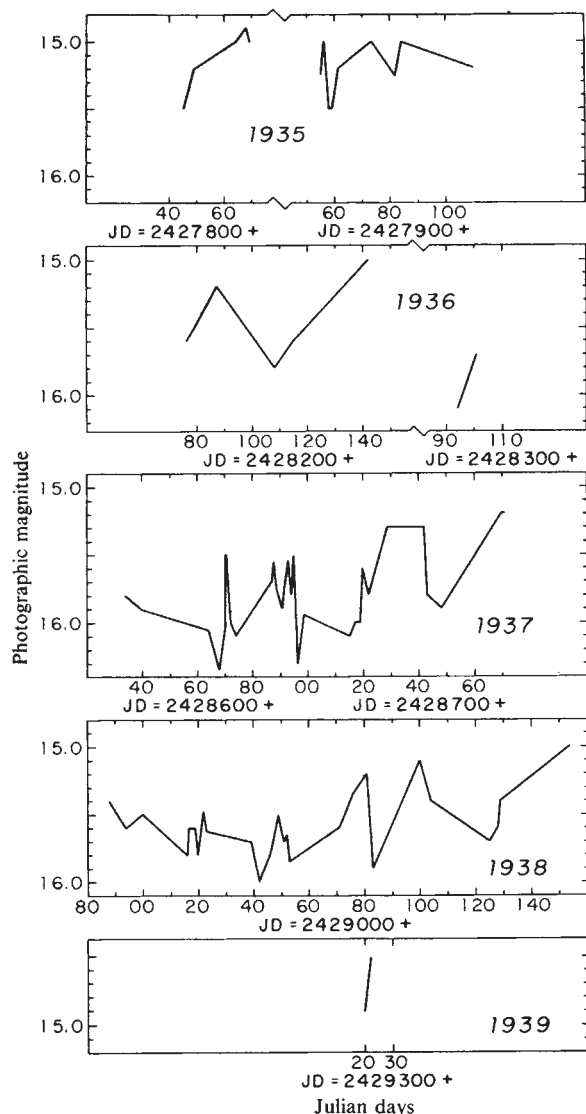


Fig. 1 Light curve of the variable star AP Lib.

the variable star AP Lib by Ashbrook⁸. The position of the star measured on the Palomar Sky Survey agrees with the first optical position given by Bolton *et al.*⁷ and with the various radio positions. A second optical position given later by Bolton⁹ is clearly in error.

Table 1 Positions of W Com and ON 231

	R.A. (1950.0)	Dec. (1950.0)
W Com	12 h 19 m 01.2 ± 1 s	+28° 30' 26" ± 10"
ON 231	12 h 18 m 58.2 ± 2 s	+28° 29' 24" ± 30"

The measurements by Ashbrook cover the period from February 1935 to March 1939, and the resultant light curve is shown in Fig. 1. The magnitude usually lies between 15 and 16, with limiting values of 14.5 and 16.4. There are periods of very fast and chaotic variations; variations up to 0.8 magnitude on a time scale of a few days are common. For some nights, several plates a few hours apart are available and yield variations of 0.7 magnitude in 5 hours on June 8, 1937, and 0.6 magnitude in 3 and 5 hours on May 3 and 4, 1938. In addition to these measurements, visual inspection of fifty-three patrol plates at Harvard College Observatory covering the interval from February 1939 to July 1951 shows high activity in July 1941, July 1944, and May 1945. The maximum brightness seems to reach $m_{pg} \approx 14$ in 1944.

The very fast variations of AP Lib resemble those of the peculiar object BL Lac, which are unique in that they are more rapid than those of any QSO or Seyfert galaxy. The amplitude of the variations of AP Lib is somewhat smaller^{10,11} than those of BL Lac, but the observations are not as many or as closely spaced as in the case of BL Lac. VRO 42 22 01 has no measured low frequency component, but a spectrum which always increases toward high frequencies. The flux of PKS 1514-24 is constant (about 2 flux units) from 1,410 to 5,009 MHz (refs. 12 and 13). There is a strong discrepancy between the 408 MHz flux measured at Parkes (5.3 flux units, ref. 12) and at Molonglo (1.9 flux units, ref. 14). The Molonglo flux is likely to be more reliable¹⁵, thus giving for PKS 1514-24 a flat spectrum over the observed frequency range. On the other hand, the disagreement about the 408 MHz flux could be an indication of radio variability.

Assuming that the extinction at the galactic latitude of AP Lib ($b_{II} = 28^\circ$) is 0.6 magnitude, and the mean flux density of VRO 42 22 01 is 6 flux units at 2,695 MHz, then the ratio of optical to radio (at 2,695 MHz) luminosities is about two times greater for BL Lac.

Photometric and spectrographic studies, as well as a search for radio variability, are needed to establish the properties of AP Lib and its possible similarity to BL Lac.

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Interplanetary Lyman-Alpha: Theory and Experiment

DISCUSSIONS of the results of the Vela 4 scattered Lyman- α experiment¹ by Bowers² and Blum and Fahr³ contain errors and misinterpretation which we believe should be pointed out promptly. The use by both authors of "Vela 7" to refer to the Vela Launch 4 spacecraft also leads to confusion because the experimental data have been published as Vela 4 data.

Bowers asserts that Chambers *et al.*¹ imply that the source of the Lyman- α radiation observed is a shock front which is luminous at Lyman- α . Actually ref. 1 clearly states that the observed radiation is assumed to be solar Lyman- α , resonantly scattered from neutron hydrogen, and that the asymmetry in the observed radiation is related to asymmetry in the distribution of the scattering hydrogen. Although Blum and Fahr do not mention Lyman- α radiation in the publication⁴ referred to by Bowers, their further published work⁵ also attributes the observed Lyman- α to scattering of radiation originating at the Sun.

Bowers plots the region of observation of the Vela 4 experiment on his galactic line-of-sight plot (partly reproduced in Fig. 1), then indicates the location of the maximum in the observed Lyman- α radiation and notes that the maximum falls on the Milky Way. The path shown by Bowers, however, does not apply to the Vela 4 Lyman- α observations. The detectors in question were mounted on the centre column of the spacecraft and aligned to look outward along the spin axis. The satellites were Earth-oriented by maintaining the direction of the spin axis towards the centre of the Earth, and the observed region of the sky is mapped by the orbit of the satellite. The correct observed region is approximately 15° on either side of the orbit as shown in Fig. 1. Also, the conveniently localized maximum flux region shown by Bowers has no basis in fact. The broad maximum of the Vela 4 data, an example of which is shown in Fig. 2, occupies at least one third of the orbit. There is no obvious indication of crossing the Milky Way in any of the Vela data except, in some cases, a minor peak of amplitude about 15 Rayleigh near $\alpha = 105^\circ$. The peak is consistent with ultraviolet measurements of ϵ CMa by Stecher⁶. Further evidence of the lack of enhanced emission in the direction of the Galaxy is provided by the Mariner 6 data⁷ and the OGO 5 measurements of Thomas and Krassa⁸.

In attempting to relate theoretical calculations of the intensity of scattered Lyman- α radiation observed from the Vela orbit with experimentally obtained plots, Blum and Fahr³ have treated the Vela 4 points as mean values to fit the smooth curve shown in Fig. 3; actually the plotted points are lower step points. The Vela 4 Lyman- α instrument output is telemetered in rather coarse digital form so that the observed signals lie in

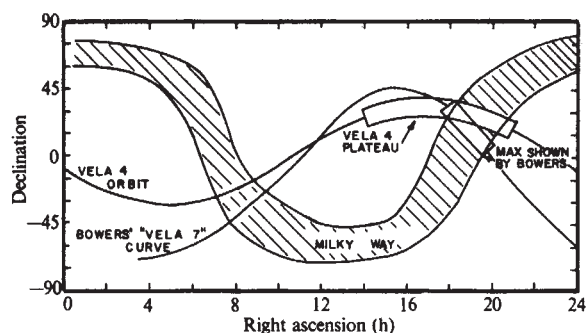


Fig. 1 The "Vela 7" portion of Bowers's Fig. 1 (ref. 2) with the Vela 4 orbit and plateau shaped maximum region added.

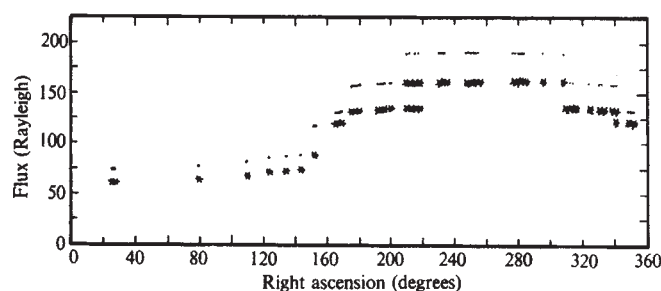


Fig. 2 Scattered Lyman- α data for the June 6, 1967, to June 10, 1967, orbit of spacecraft 4A. The lower step ($\star$) to upper step ($\bullet$) interval is a band in which the observed flux lies.

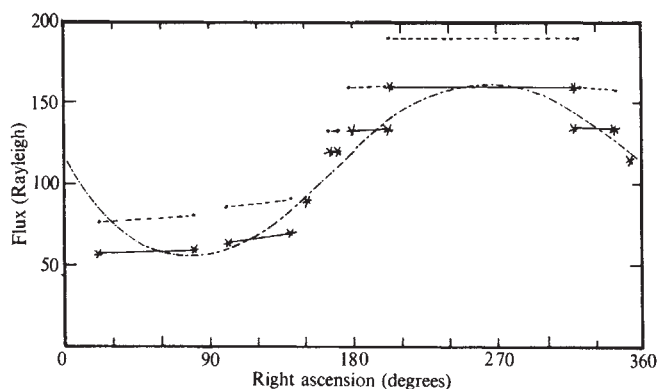


Fig. 3 The curve fit to the original Vela 4 points ($\star$) by Blum and Fahr³ with upper step points added ($\bullet$).

an interval between possible output steps. The lower step has been arbitrarily used because the experimental error in the Lyman- α experiment is large compared with the interval between steps. Much of the error, however, originates in the determination of the flux used for laboratory calibration so that the principal part of the error is proportional to the flux observed. Thus the shape of the plotted data is more significant than its absolute value, and a curve passing between a pair of points more accurately represents the data. If the curve of Fig. 3 were made to fit the data in the manner just described, the shape would be somewhat different from that depicted by Blum and Fahr and the correlation between theoretical predictions and measurements may not be as good as it appeared.

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Re-evaluation of the Palaeogeographic Argument for an Expanding Earth

EGYED¹ has inferred that the Earth was expanding at a mean rate of 0.5 mm yr^{-1} during Phanerozoic time. The evidence on which he based this conclusion was taken from two series of palaeogeographic maps showing the distribution of land and sea since the early Cambrian, by Strakhov and H. and G. Termier respectively. With the aid of a planimeter Egyed estimated the percentage of continental areas covered by the sea for successive geological periods and was able to demonstrate clearly a secular decline since the early Palaeozoic with the more or less regular downward trend having superimposed on it a series of shorter phase oscillations.

As the volume of ocean water is hardly likely to have declined

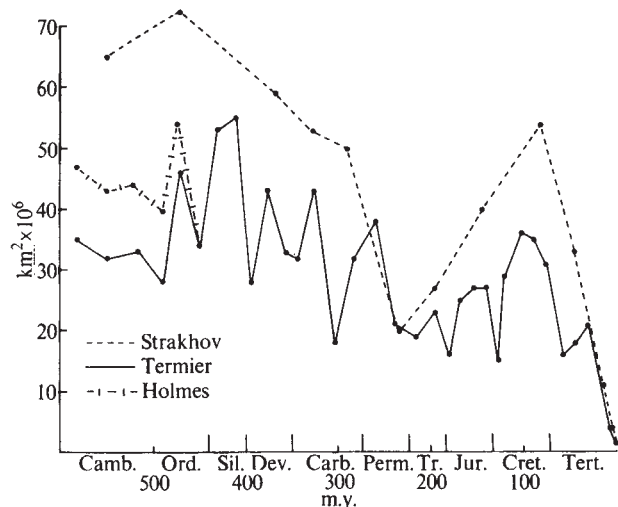


Fig. 1 Areas of continents covered by sea in Phanerozoic time.

during Phanerozoic time, and might indeed have been augmented by a small percentage, Egyed argued that the facts could only be explained by a gradual increase in the Earth's volume, so that ocean water drained more or less progressively from the continents into the growing oceans.

The essential palaeogeographic facts are not in dispute. As plotted in Fig. 1, the two groups of data, though differing appreciably, are in accord as regards the decline through time of areas of the continents inundated by seawater, the only notable (but temporary) reversal of this general trend being in the late Cretaceous. Of the two sets of data, that of the Termiers, based as it is on more numerous time intervals, gives a more accurate idea of the oscillatory nature of the change. The modification of the Cambro-Ordovician data proposed by Holmes² is accepted here. Apart from the areas of metamorphic rocks in Africa and elsewhere that were probably marine sediments originally, missed by the Termiers, it should be borne in mind that the older the rocks the greater is the likelihood of their having been removed by subsequent erosion or deeply buried beneath younger sediments beyond the limits of intensive drilling. For these reasons the amount of the secular decline is more likely than not to be underestimated in most palaeogeographic maps.

Both Egyed and those who have accepted his argument²⁻⁴ have failed to consider an alternative explanation of the facts, that the average thickness of the continents has increased slightly through the course of the Phanerozoic. The isostatic uplift consequent on such increase would inevitably have the effect of reducing the area of marine inundation. There are grounds for believing that changes of this sort might, in fact, have taken place.

In the first place, many ancient shields have persisted for millions of years as positive areas and sediment sources. Maintenance of relief following removal of sediment would naturally result from isostatic adjustment, but obviously this could not persist indefinitely because the shields would achieve isostatic stability with low relief and relatively thin crust. In fact such shields often have crustal thicknesses greater than the average⁵, even though metamorphic rocks of granulite facies may be exposed at the surface. Extensive tracts of the Archaean shields consist of the so called Greenstone Belts, which are believed to signify appreciably thinner crusts in early Pre-Cambrian times, perhaps only 12–15 km. This is based on various data, including the chemistry of the metabasalts and comparisons with present day island arcs⁶. The implication is that the shields have undergone a progressive "sialic underplating" since the early Pre-Cambrian as a result of slow differentiation in the underlying mantle.

In the second place, geochemical and other data suggest that the andesites of island arc complexes and the associated

granitic batholiths in regions such as western North America originate primarily by differentiation of basaltic magmas arising from descending slabs of ocean floor along subduction zones, with only limited contamination by continental crustal materials^{7,8}. At the rate of thickening implied by volcanicity since the Miocene, the whole of the Japanese crust could have been generated in about 3×10^9 yr⁷. This process provides a ready mechanism for creating continents which is consistent with the tenets of plate tectonics. Many former geosynclinal regions emerged from the sea following major orogenies and became incorporated into cratons bordering the ancient shields and so something more than lateral accretion is involved. Sutton⁹ has incorporated such facts into his notion of chelonogenic cycles, whereby tectonically mobile belts characterized by flysch-type marine sedimentation and volcanicity are transformed into stable shield areas or cratons, on which only continental or shallow marine sediments were laid down, the latter often only at the margins. The conversion of the Caledonian geosyncline of the North Atlantic region into the "Old Red Sandstone Continent" in Devonian times is a good example, because it persisted as a positive area until well into the Mesozoic, when it was disrupted by the drifting apart of North America and Europe.

An approximate estimate of the amount and rate of thickening of the continental crust can be obtained by considering estimates of the changes in relative position of sea level in the past 600 million yr or so. Egyed¹ estimated that sea level in Cambrian times was about 500 m higher than today, if Strakhov's data were to be relied upon, or 275 m higher in the middle Ordovician, using the Termiers' data. Kuenen¹⁰ was able to show from the world hypsographic curve that under present conditions a eustatic rise of 100 m would flood about 1/4 to 1/5 of the continents and a rise of 200 m nearly 1/3. He considered that the present continents have abnormally high relief and so thought that at most periods in the past a 100 m rise would flood more than 1/3 of the continental area. The palaeogeographic maps available to Kuenen seldom showed more than 1/3 of the continents submerged, and he concluded that the biggest transgressions did not involve eustatic rises of more than 200 m.

These figures suggest that the Termiers' data might be more accurate than those of Strakhov. An independent check on the maximum area covered by the Jurassic seas¹¹ also gives a figure (24%) much closer to that of the Termiers (27%) than Strakhov (39.5%).

If we take 300 m as a figure of the right order of magnitude for the amount of secular lowering of sea level since the early Palaeozoic, this would be accounted for by an average thickening of the continental crust of perhaps twice this amount. Such a figure implies an increase of only about 1 mm in 10^3 yr, with no more than about 2% of the present average thickness of the continental crust being added in the last demi-eon. Modest, almost negligible, change of this kind could be accounted for readily by the processes previously discussed. With such a plausible alternative interpretation being available the simple palaeogeographic argument for an expanding Earth cannot reasonably be sustained.

The shorter phased eustatic oscillations superimposed on the gradual lowering of sea level, given crude and oversimplified expression in Fig. 1, are to be accounted for in other ways. Whereas glacially controlled eustatic changes dominated Pleistocene palaeogeography it is doubtful whether the melting and freezing of polar ice played more than a minor role further back in time. Thus even the well documented late Palaeozoic Ice Age of the southern continents had no clearly perceptible large scale effect. On the contrary, the apparent disappearance of the ice sheets at the close of the Palaeozoic coincided with a pronounced marine regression rather than a transgression. The dominant control was much more likely to have been variations in the cubic capacity of the ocean basins due to the uplift and subsidence of mid-oceanic ridges and trench systems¹². Evidence is now accumulating to suggest that uplift

and subsidence of mid-oceanic ridges correlate respectively with increases and decreases of seafloor spreading rates¹³. The more fundamental control is presumably temporal variations in heat flow from the mantle.

The two most notable departures from a more or less gradual lowering of relative sea level in the Phanerozoic are the pronounced regression of the late Permian and early Triassic, and the equally striking transgression of the late Cretaceous. This is brought out more clearly in the less accurate Strakhov curve of Fig. 1. In fact both curves probably over-estimate by a considerable margin the area of continent covered by the sea at the close of the Palaeozoic, which was closer to the minimal values of the Pleistocene than anything before or since. Both phenomena probably relate to significant plate movements. Valentine and Moores¹⁴ have argued that the suturing of Palaeozoic continents to form "Pangaea II" would be a sufficient cause of regression in the late Permian. With regard to the late Cretaceous, a major transgression could have been produced by one, or both, of the following factors: an acceleration of spreading rate or an increase in the length of the oceanic ridge system as significant dispersal of both northern and southern sectors of Pangaea got under way. The probable timing of continental breakup¹⁵ suggests the latter cause to be sufficient.

I thank Dr E. R. Oxburgh and Dr S. W. Richardson for their helpful comments.

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Laser Radar Reflexions and Downward Infrared Flux Enhancement near Small Cumulus Clouds

WE wish to report some interesting simultaneous enhancements of backscattered laser pulses and the downward infrared flux in regions of clear air surrounding small cumulus clouds. The observations were made at Adelaide, South Australia, in March 1970, and at Aspendale, Victoria, in February 1971, but at Aspendale only a radiometer was operated.

We used a ruby laser (0.694 μm) with a pulse length of 0.5 μs , and the transmitter and receiver beamwidths were 1.0 mrad and 5.7 mrad respectively¹. The field of view of the radiometer (10–12 μm) was 6 mrad and it had a minimum detectable radiance of 0.002 $\text{mW cm}^{-2} \text{sr}^{-1}$ with an output bandwidth of 0.1 Hz (C. M. R. Platt, private communication). Both instruments pointed vertically upwards and were 100 m apart at Adelaide.

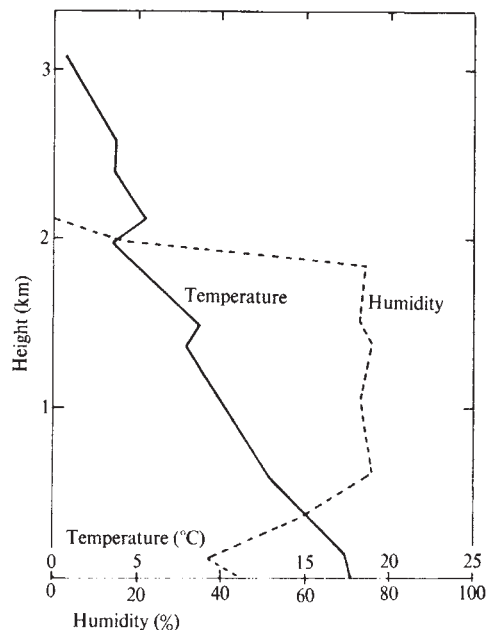


Fig. 1 Temperature and humidity profiles.

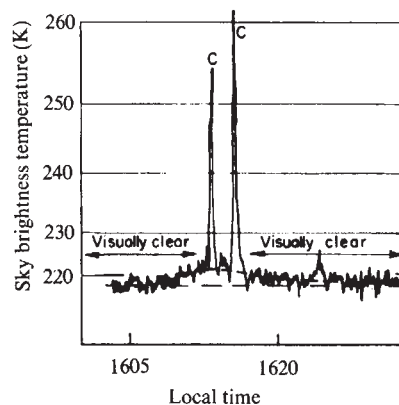
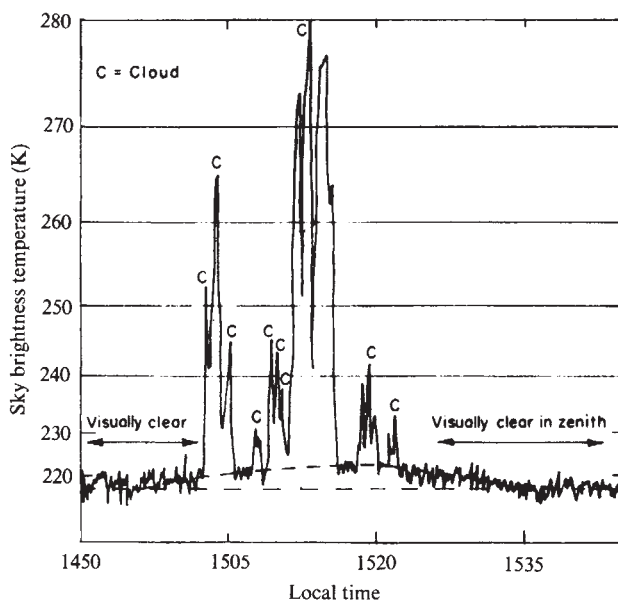


Fig. 2 Zenith sky brightness temperature (10 μm –12 μm).

In clear skies, backscattered radiation at the 0.694 μm wavelength of the laser is due to Rayleigh scattering from molecules and Mie scattering from aerosols, and the back-

scattered intensity is, of course, greatly increased when low cloud is present. On the other hand, atmospheric water vapour is responsible for the downward radiant flux at 10–12 μm when the sky is clear, although there is a small contribution from aerosols.

The weather at Adelaide was controlled by a cool southerly maritime airstream that was moist under an inversion at 2 km but dry aloft (Fig. 1). Small cumulus clouds were forming in groups and drifting over the site, but there was no rain. Conditions at Aspendale were similar.

Fig. 2 shows the infrared enhancements that occurred around groups of cumulus. The arrival of visible cloud edges (*c*) is marked by sharp increases in downward radiance, but the interesting point is the slow increase in radiance that occurs outside the visible clouds. The increased radiance persists between clouds, but reverts to the original level after the passage of a group of clouds.

Fig. 3 compares the radiometer measurements with simultaneous laser radar (lidar) observations. Aerosols must be responsible for most of the observed variation in the backscattering coefficient $I'(h)$ which is normalized to unity at 2 km. This is because between 0.4 and 2 km the molecular

backscatter would only vary by the factor 1.14 (because of the pressure change).

The enhancement of $I'(h)$ is very marked, particularly in the region of the cloud base which was estimated from the measured radiance and radiosonde data and is correct to 200 m, and it is clear that the enhancement continues in the gaps between clouds. Fig. 3 also shows reasonable agreement between the onset of the lidar enhancement and the increase in radiance. In this figure, in contrast to Fig. 2, the clouds have fairly constant radiances, indicating that they must have been optically thick.

The same pattern was observed four times on March 19, 1970, as four different cloud groups passed overhead. Fig. 4 shows another example.

The most likely explanation is that hygroscopic aerosols are taking up water and swelling under conditions of increased humidity as the air below the condensation level is lifted, and cools. That must be why the maximum enhancement of backscatter occurs near the level of the cloud base, the condensation level. Particles at 95% humidity have about twice the radius of those in the dry state, and towards 100% humidity the increases are from three to ten depending on the nature of the

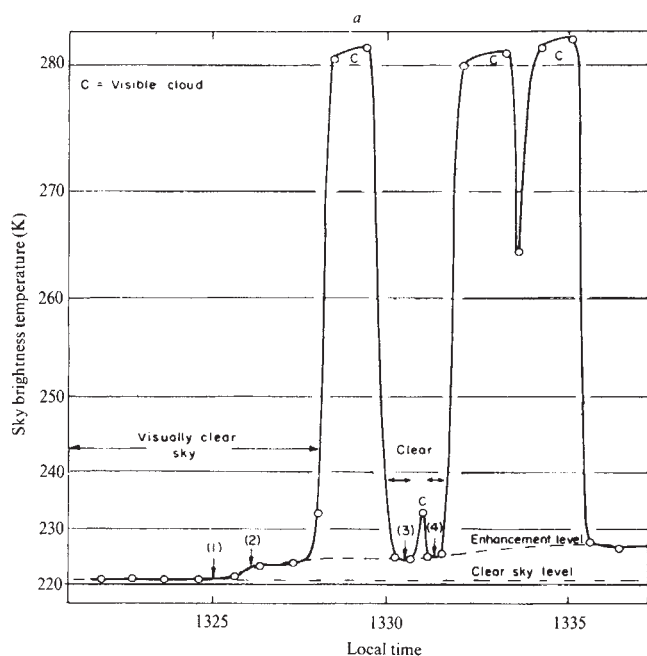
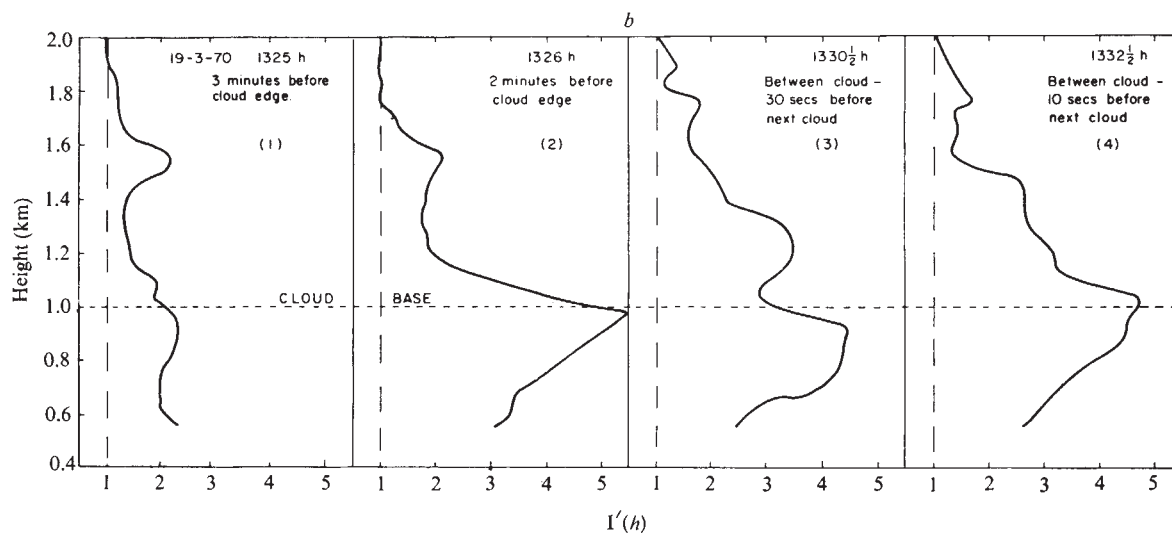


Fig. 3 *a*, Zenith sky brightness temperature (10 μm –12 μm).
b, Lidar intensity–height profiles (normalized at 2 km).



particles^{2,3}. And because the increased volume is composed of liquid water, which is highly absorbing in the infrared, the emissivity of the particles is increased. Clearly the increase in particle size and change in refractive index affect the lidar observations. Table 1 shows the relative increases in backscattered intensity predicted by Mackinnon⁴ for the case when half the aerosols in both continental and maritime distributions are hygroscopic. These results can be compared directly with experiment as the morning humidity at Adelaide was 75% at 1 km, and the increase in aerosol backscatter measured by us agrees approximately with that predicted by Mackinnon if the humidity approaches 100%.

(humidity mixing ratio) is increased, due either to entrainment of moist air or the evaporation of water droplets near the cloud edges through mixing with the surrounding air. For example, the emissivity of a layer 300 m thick would increase by 0.01 if the specific humidity changed from 5.7 g kg⁻¹ (early morning value at the cloud base) to the saturated value of 7.3 g kg⁻¹ at the cloud base temperature. In all probability the infrared enhancement is due to both swelling of aerosols and increased water vapour, but the lidar enhancement can only be due to an increase in aerosol size in this model. But there is also the possibility of an increase in aerosol density near the clouds because of updrafts. Even if the aerosols remained dry, the

Table 1 Humidity Effects on Backscattered Lidar Power from Aerosols, Hygroscopic Aerosol Radius and Infrared Emissivity at (10–12 μ m)

Relative humidity H (%)	Ratio of aerosol back-scattered power at H to that at 75% humidity ⁴		Ratio of aerosol radius at H to that at 75% humidity		Increase in emissivity E_λ	
	C	M	C	M	C	M
96	1	2	1.7	2	0.0025	0.002
97	1.3	4				
98	1.5	4.5				
99	3	5	2	3.5	0.003	0.005
100	~5	~10	3	10	0.01	0.035

Mean observed emissivity = 0.02.

C, Continental distribution; M, maritime distribution.

The corresponding change in infrared emissivity for a homogeneous layer of aerosols has been computed using similar continental and maritime distributions to those used by Mackinnon. For an optically thin layer, and neglecting scattering, the emissivity E_λ is given by $(1-\tau_\lambda)$, where τ_λ , the optical thickness, has been computed from Mie's theory. Computed values of E_λ for a 300 m layer are shown in Table 1 and compared with the mean observed value. It can be seen that the theoretical emissivity approaches the measured emissivity for either distribution if the relative humidity approaches 100%. The lidar results seem to favour the continental distribution although the actual value of $I'(h)$ at the condensation level is greater than that shown because of the low laser pulse resolution. At a coastal site such as Adelaide one would expect the aerosols to be a mixture of continental and maritime types, and the theoretical computation certainly gives order of magnitude agreement with observation.

It should be pointed out that the infrared emissivity will also increase near the clouds if the specific humidity of the air

density increase might account for the observed increase in lidar backscatter intensity, but the infrared emissivity increase would be considerably less than that observed. (This was verified by combined observations of aerosols in dry conditions.) Thus it seems that the aerosols must also swell to account for the large emissivity change.

Table 2 Average Extent of Lidar and Radiometric Enhancement around Small Cumulus Cloud Groups

Date	Windward (km)	Leeward (km)
March 19, 1970	1.3 (4)	—
February 10, 1971	3.4 (3)	5.8 (2)

Figures in brackets give number of observations.

Table 2 summarizes the spatial extent of the observed enhancement. The phenomenon carries with it the implication that in

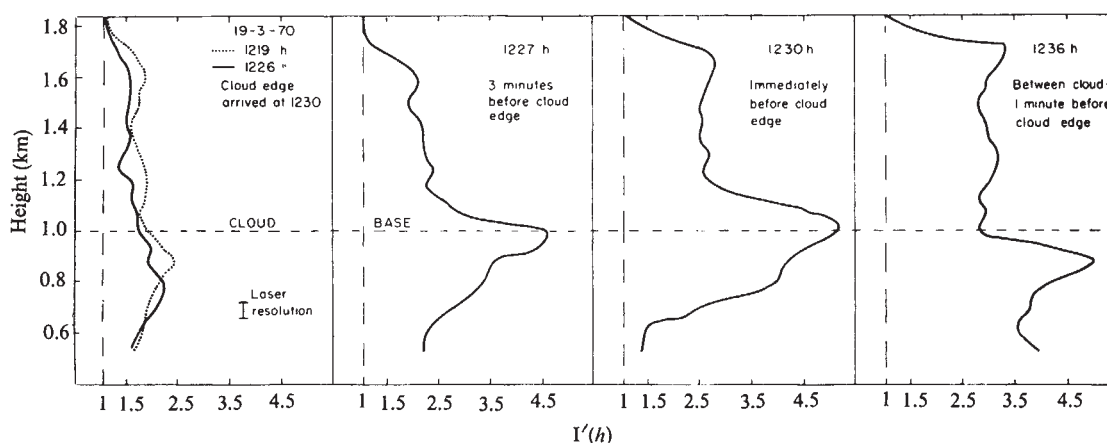


Fig. 4 Lidar intensity-height profiles (normalized at 2 km).

some cases there is a broad region of uplift in and surrounding groups of small cumulus clouds. So the clouds seem to be fed from below over a broad region.

On a few occasions, however, the effect was absent, or much reduced. This may be because when cumulus clouds are decaying the updraughts are replaced by downdraughts⁵.

It is interesting that Wilkins *et al.*⁶, using a wavelength difference technique, observed haloes around cumulus clouds in visible and near infrared photographs taken from Apollo 9 (SO-65). The haloes were also evident in a photodensitometer profile across a cloud bank image, and extended 1 km from the cloud edge. It seems likely that these authors and ourselves are observing the same phenomenon.

We thank Dr W. G. Elford for cooperation in making these measurements possible.

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Effect of Time on the Mechanical Behaviour of Failed Rock

WHEN a rock specimen is compressed from an initial intact state to complete collapse, the failure locus can be traced out on a force-displacement diagram as shown by the solid line in Fig. 1a. Until recently, this curve was followed in experiments only to the point where the compressive strength was reached; the rock then disintegrated violently. This catastrophic failure is caused by the elastic unloading of the testing

machine after the peak of the force-displacement curve and is not an intrinsic property of the rock. The elastic strain energy released by conventional "soft" testing machines exceeds the amount of energy required to break the rock and the excess energy is liberated in the form of kinetic energy. Uncontrollable failure can be avoided by the use of "stiff" testing machines which store very little strain energy¹⁻⁶, or by closed loop servocontrolled testing machines which can reduce the load faster than the specimen breaks⁷⁻⁹.

When the force-displacement curve is scaled by the cross-sectional area and length of the specimen, a complete stress-strain curve is obtained. For the design of structures in rock it is important to establish whether this curve is a material property or varies with the experimental conditions. In particular, the effect of time on the complete stress-strain curve must be established. It is known that the part of the stress-strain curve of rock before the peak is time dependent¹⁰⁻¹⁵ and it is likely that the section after the peak is even more sensitive to time because the specimen is in a continual state of progressive collapse.

All research on the time dependent behaviour of rocks before the peak of the stress-strain curve has been carried out with reference to strain rate and creep. The only research on the effects of time in the region of the curve after the peak¹⁵ was concerned with the effect of strain rate on the complete stress-strain curve for sandstone. Bieniawski¹⁵ found that the descending side of the stress-strain curve became steeper as the strain rate increased. He also attempted to study the creep of failed rock but could not achieve a constant stress condition because the rock deformed along the elastic unloading characteristic of his testing machine. In soft or stiff mechanical testing machines, only a limited range of boundary conditions can be achieved precisely. This is presumably the reason why the effects of strain rate and creep have been studied exclusively. With the advent of closed loop servocontrolled testing machines, it is now possible to achieve almost any required boundary conditions and all aspects of the effect of time on the complete stress-strain curve for rock can be studied.

In a servocontrolled testing machine the specimen is loaded by a hydraulic actuator and the actuator pressure is adjusted by means of a servovalve. A feedback signal (t) representing some experimental condition of the specimen is electronically compared with a programme signal (p) representing the required condition. If a difference exists, an error signal operates the servovalve and the pressure on the rock is adjusted until t coincides with p . The mode of operation of the closed loop is shown in Fig. 1b. The comparison of t and p can be made 10,000 times per second and the response time of the system can be as low as 0.002 s. If a constant stress rate is

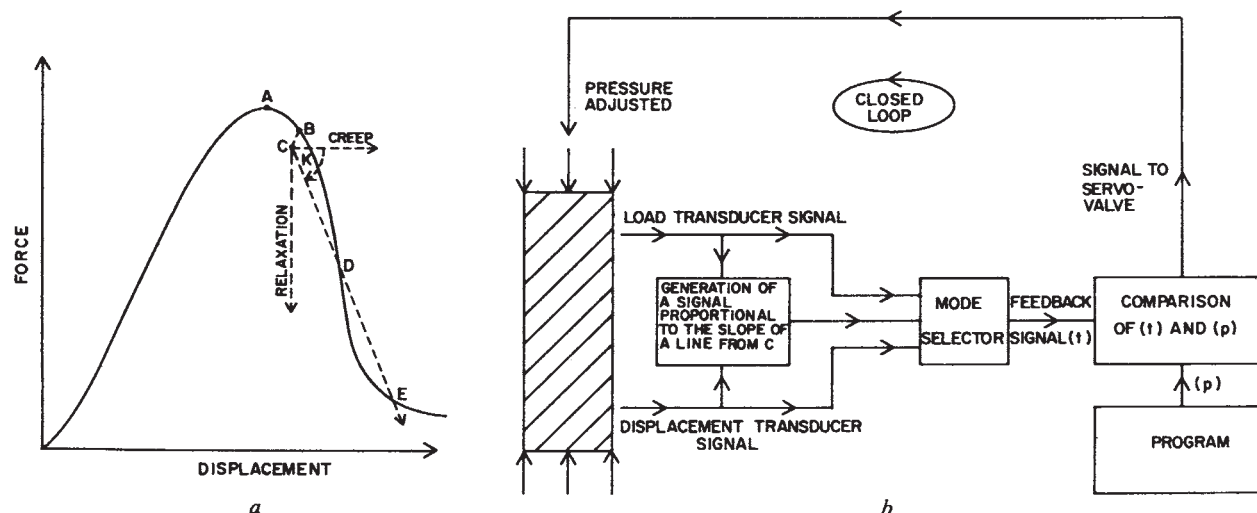
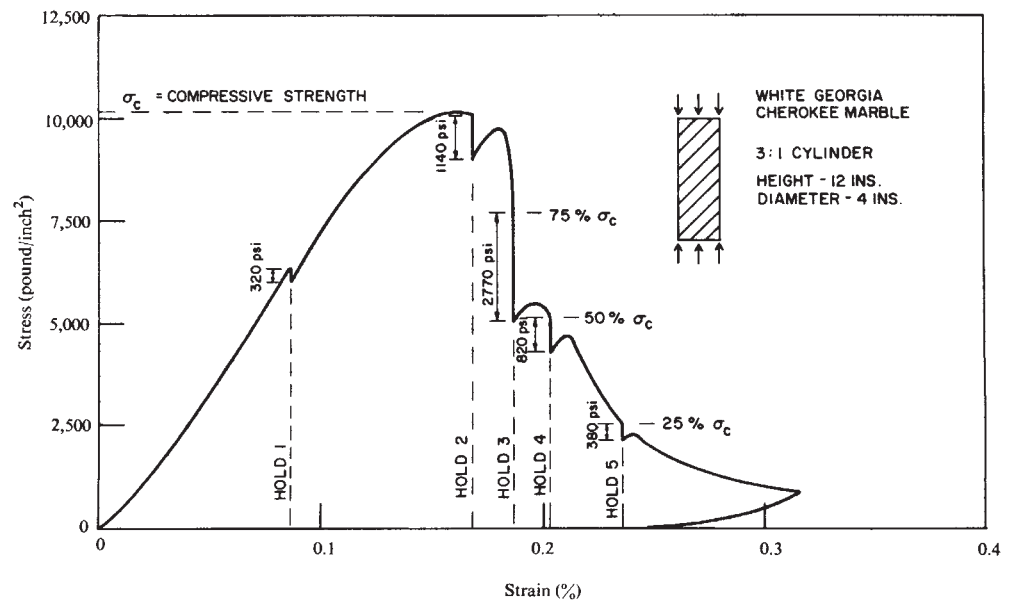


Fig. 1 a, Typical complete force-displacement curve for rock; b, closed loop servocontrolled system.

Fig. 2 Stress relaxation in failed marble.



required, the feedback signal is generated directly by a load cell and p linearly increases with time. The rock will fail very violently at the peak of the stress-strain curve in these conditions because the machine attempts to increase the stress as the rock is breaking. When a constant strain rate is required, the feedback signal is generated by a displacement transducer and again compared with a linearly increasing programme signal. As the specimen displacement is linearly increased by the operation of the closed loop, the associated stress rises to a maximum and then slowly falls as the complete stress-strain curve is traced out. To study creep, the programme is stopped at any required stress level with the feedback signal corresponding to the load. A constant stress level is then achieved by the operation of the closed loop. To study relaxation, the programme is stopped at any required strain value and the feedback signal then corresponds to the specimen displacement.

An example of stress relaxation is given in Fig. 2 which is a complete stress-strain curve for white Georgia Cherokee marble loaded in uniaxial compression. The specimen was strained at room temperature at a constant rate of $1.5 \times 10^{-6} \text{ s}^{-1}$ except for the five points on the displacement axis at which the displacement was held constant for one hour. The first displacement hold was made during the apparently linear ascending portion of the force-displacement curve. The remaining four holds were made just after the peak and at 75%, 50% and 25% of the compressive strength. The amount of stress relaxation along each of these constant displacement lines is shown in Fig. 2. When the strain is increased after a hold, the curve seems to rise to a continuation of the original curve, indicating that the descending part of the complete stress-strain curve is not affected by this type of variation in the strain increase. The same result was obtained by Bieniawski¹⁵ for time deformation of sandstone along the 1,110 MN/m unloading characteristic of his stiff testing machine. The maximum amount of stress relaxation occurred at the displacement corresponding to 75% of the maximum stress. This suggests that stress relaxation will be a maximum where the stress-strain curve is steepest, that is, when the relaxation line is closest to the failure locus.

Both creep and stress relaxation are phenomena associated with very specialized applied boundary conditions. In practice, for example, a mine pillar left for a period of time, the rock is subjected to neither a constant load nor a constant deformation but to a condition dictated by the unloading of the surrounding rock. To study time-dependent deformation along the line of arbitrary slope $-k$, both the load and displace-

ment transducer signals must be used to generate the feedback signal. If the experimental force-displacement coordinates are above the line CE in Fig. 1a, the error signal should cause the servovalve to reduce the pressure. Conversely, if the coordinates are below CE , the pressure should be increased. The operation of the closed loop with a slope feedback thus allows a study of time deformation along any such line. If a specimen is strained to a point B on the complete stress-strain curve and then unloaded slightly to a point C as shown in Fig. 1a, the closed loop operation can be modified to study any type of time dependent behaviour. A constant stress condition would result in creep back to the failure locus. A constant displacement condition would result in stress relaxation to a safer condition (provided the stress-strain curve increased monotonically). If the machine was programmed to follow the line CDE , the specimen could deform to the failure locus at B , drop quickly to E and then stabilize at some lower stress value.

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Optical and Acoustic Detection of Ball Lightning

THE recent controversy about ball lightning¹⁻⁴ stimulates me to report an observation of such an event, which occurred in September 1963.

This event occurred at approximately 11 a.m. in the entrance hall of the Plöckenhaus, a restaurant below the Plöckenpass in the Carnic Alps (Austria). Students from a field trip of the Geology Department of the Universität Heidelberg were staying inside this restaurant because of a thunderstorm with heavy rain. Some of the students were sitting in the guest room and some were standing in the entrance hall. The outside doors of the house were open. The door between the guest room and the entrance hall was, as I remember, closed. The floor of the entrance hall consists of heavy limestone plates. Also in the entrance hall was a St Bernard dog, lying on the floor.

Suddenly, through the open outside doors, a whitish yellow ball appeared just above the floor. It was slightly larger than a tennis ball, and its speed was about that of a walking person. The light ball moved about 2 m in the direction of the dog and finally exploded with a loud bang, like a gun shot. The dog jumped away as it saw the moving light ball. All the people in the entrance hall (about five) saw the ball and heard the bang. People from the guest room came out to ask what happened, because they had heard the bang. I do not remember feeling any radiation heat from the bang, nor smelling anything after the explosion. We, the bystanders, explained this event as a "Kugelblitz" (ball lightning).

The optical observation by several people, the reaction of the dog, and the acoustic perception of the people in the adjacent room are hard to explain by the optical illusion hypothesis for ball lightning.

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On Fireballs

THE recent discussions on fireballs prompt me to put on record that a few years ago I saw one when staying at a villa near Malcesine on the east shore of Lake Garda in Italy.

A bad thunderstorm had been raging and, because I still harbour a childish phobia about looking at lightning, I had not been looking out of the window. But as the storm slackened and was passing away, I did look out and saw a brilliant ball of white fire "sitting" still on one of the pylons carrying power lines along the east side of Garda, only a few hundred yards from our villa. It sat on one of the horizontal cable bearing cross-beams, and its diameter was less than the vertical distance between the cross-beams. There could be no question of my, or my friends, seeing an after-image of lightning, particularly as I never look at lightning myself.

We looked at the fireball for some time, but as it did nothing but sit still, we lost interest; when we looked out later, it had disappeared.

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BIOLOGICAL SCIENCES

Crystal Structure of the Visual Chromophores, 11-*cis* and all-*trans* Retinal

THE visual chromophores, 11-*cis* and all-*trans* retinal (Fig. 1), are present in both rod and cone cells of vertebrate retinas and in the corresponding organs of insects and crustacea¹. The isomerization of the 11-*cis* isomer to the *trans* form may be the only step in the visual process which is initiated by light; several succeeding changes in the visual pigment are known to occur in the dark^{2,3}. In spite of their importance in vision, the conformation of the chromophores have not been determined. Their three dimensional configurations in the crystalline state are presented in this communication.

Theoretical chemists have attempted to explain the stability and predict the structure of 11-*cis* retinal. Recent calculations predict a very slight difference in energy for the two isomers⁴⁻⁶, which is consistent with thermodynamic measurements⁷. However, the structural models proposed differ from the conformation found in the crystal*. In these calculations, bond distances and angles characteristic of *trans*-carotenoids were assumed, and various conformational possibilities were explored by varying torsional angles about bonds. The discussion below indicates that several small but significant distortions in bond angles may be critical in the folding process.

The collection of X-ray diffraction data requires a relatively stable crystal. Fortunately, crystals of the *cis* isomer are more stable than its solutions, which easily isomerize at room temperature. Preliminary attempts to collect data indicated that a special environment was needed. The four-circle goniostat of an automatic diffractometer was enclosed in a light-tight container maintained at 16–17° C and continuously purged with dry nitrogen. In this manner, it was possible to collect 2,345 integrated intensities from one freshly synthesized crystal over about 4.5 days. The data were corrected to compensate for a 20–25% downward drift in monitored intensities. A total of 2,995 independent reflexions was also collected from the *trans* isomer.

(The 11-*cis* isomer formed twinned crystals in space group $P2_1/c$ with $a = 7.540$, $b = 10.666$, $c = 22.102$ Å, $\beta = 95.19^\circ$, and four molecules/cell. The *trans* isomer crystallized in $P2_1/n$, with $a = 14.961$, $b = 8.279$, $c = 15.316$ Å, $\beta = 104.87^\circ$, and four molecules/cell. Both isomers were crystallized from petroleum ether (Baker Analyzed Reagent, boiling point 36–37.5° C). Crystallization was initiated at 5° C and completed at –20° C. The *cis* isomer was synthesized when needed from commercially available *trans* retinal, using the methods of refs. 8 and 9.) The unit cell parameters found for the *trans* isomer agreed with those reported by Kuwabara *et al.*¹⁰.

Approximate positions for the carbon and oxygen atoms of the two isomers were established by the symbolic addition procedure¹¹. Refinement of the atomic positions and their apparent anisotropic thermal parameters was accomplished with standard least squares refinement programmes. For both isomers, the hydrogen atoms of the chain and most of the methyl hydrogens were located in difference maps. Based on cell data the agreement factors, that is the crystallographic R-factors, are at present 12.6% (11-*cis*) and 9.9% (*trans*). Further refinement and full hydrogen determinations seem feasible, and the final results, along with more crystallographic detail, will be published later.

The suggested three dimensional structures of the two retinals are presented in Fig. 1. The all-*trans* retinal chain is planar,

* Since our manuscript was prepared an article has appeared¹⁶ which does theoretically propose a ground-state configuration for 11-*cis* retinal, closely resembling that reported here. The torsional angles of the chain are predicted by Honig and Karplus, and these parameters effectively define the shape of the molecule. Their torsional angles are all within 11° of our experimental values.

with the six-membered ring inclined to the plane of the chain. The inclination is given by the torsional angle of the 5-6-7-8 segment which measures 59° (the torsion angle of a planar *trans* conformation is 180° , and of a planar *cis* conformation is 0° ; torsional angles are defined in ref. 12). This arrangement is typical of carotenoids, and the *trans* isomer does not differ significantly in any details from previously reported *trans* carotenoids¹³.

The *cis* isomer is similar to the *trans* at the ring-chain junction; the ring torsion measured at atoms 5, 6, 7, and 8 is 40° . The chain segment from atoms 6 to 13 is essentially planar; this is contrary to the theoretical predictions mentioned earlier. The proposed models were nonplanar in this region, with torsions at the 10-11 or the 11-12 bonds ranging from 10 to 50° . The X-ray analysis indicates rotations from planarity of 8° , 4° , 0° , and 3° at the 8-9, 9-10, 10-11, and 11-12 bonds, respectively. The planar environment at the 10-11 bond corroborates a prediction of Patel¹⁴, based on NMR observations. There is, however, an unexpectedly large torsion at the 12-13 bond, so that the atoms from C(12) to the terminal oxygen define a distinctly separate planar segment. The torsion at bond 12-13 is 39° from an *s-cis* conformation; therefore, the planar segments attached to this bond are rotated 141° from the *trans* conformation. As a result, the C(13)=C(14) and the C(15)=O double bonds are no longer parallel to the conjugated double bonds of the other segment. The best planar representation (structural formula) for this configuration is illustrated in Fig. 2, which also presents the bond distances of the two isomers. The corresponding bond distances of the chain are similar for the two isomers, even near the bend in the chain.

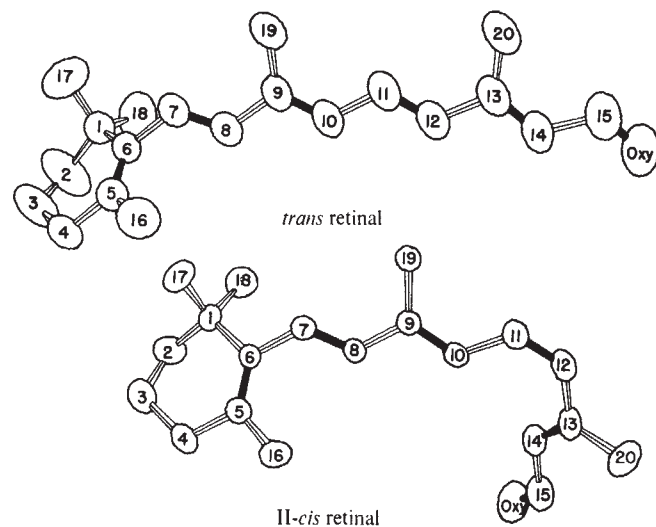


Fig. 1 Structures of the two retinal isomers. The ellipsoids drawn at each atom position indicate the relative magnitudes of the apparent thermal vibration amplitudes of the atoms. The solid black bonds represent the double bonds of the molecules.

Distortion of the bond angles of the *cis* retinal chain was noted at three points. The bond angles at atoms 11, 12, and 13 are 127.3 , 130.3 , and 120.6° in the *cis* isomer; the respective angles in the *trans* isomer are 121.6 , 124.5 , and 116.6° . The *cis* retinal has two short intramolecular approaches: atom 10 is 3.18 Å from atom 13, and 3.23 Å from atom 14. These atoms would approach more closely, and presumably would repel one another strongly, if the *cis* isomer were constrained to the bond angles characteristic of the *trans* isomer. Such a constraint is normally assumed in theoretical molecular folding calculations.

The problem of the conformation of the 11-*cis* retinal in solution remains. Large changes with temperature in the ultra-

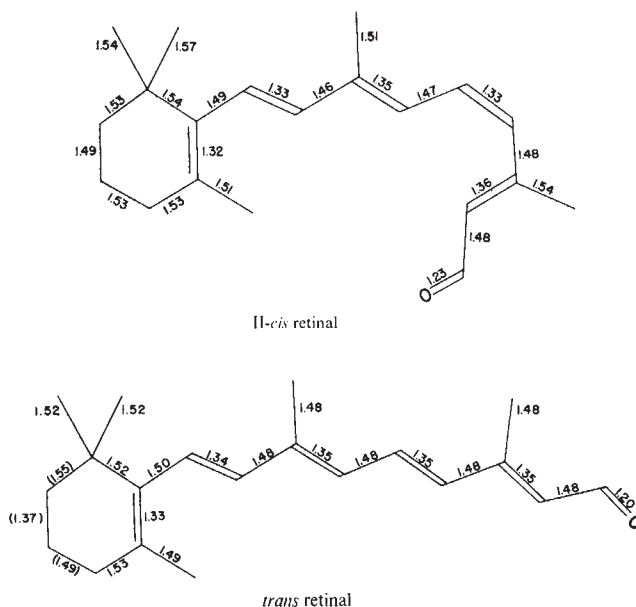
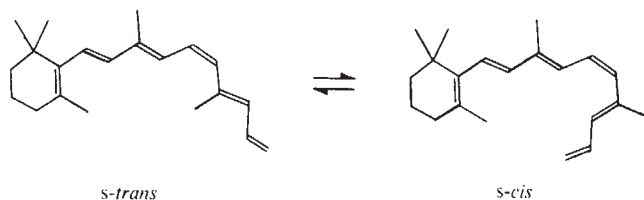


Fig. 2 Bond distances for the two retinal isomers. The planar chain of the *trans* isomer is drawn to scale and displays a slight curvature; this has been noted in other reports on carotenoid structures¹³. The bond distances in the ring of *trans*-retinal, which are enclosed in parentheses, represent the average values of a disorder. A ring puckering disorder was noted in canthaxanthin¹³ in the C_2-C_3 region, and a similar disorder is indicated for the *trans* isomer.

violet spectrum suggest an equilibrium involving more than one form of retinal. Torsions about the 10-11 and/or 12-13 bonds were discussed previously¹⁵ to explain the spectral data. At that time, torsions about the 10-11 bond were preferred. One of us (W. S.) has since examined additional spectroscopic evidence, and now considers two conformations, differing by a large torsion about the 12-13 bond, to be the most probable forms in solution, with the *s-trans* form prevailing at lower temperatures (77 K).



The labels *s-trans* and *s-cis* are approximate; in each case, twists away from these planar forms would be expected. A more detailed report on the spectroscopy of the retinals will be published elsewhere.

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Optical Activity of LSD-DNA Mixtures

REPORTS on the fluorescence-sensitive binding of d-LSD (d-lysergic acid diethylamide), l-LSD and their 2-bromo derivatives to calf thymus DNA¹, and on changes induced in DNA circular dichroism by LDS-25 (ref. 2), have led to the belief that it is the intercalation of the drug in DNA that causes the chromosomal damage³⁻⁶ and abnormalities in human offspring⁷ that have been associated with LSD. Our studies of circular dichroism, however, have failed to show that LSD has any effect on DNA conformation.

To begin with, the circular dichroism (CD) of d-LSD, l-LSD and d-2-bromo-LSD at high concentrations does not conform with Beer's law: at concentrations greater than 0.02 mg/ml., for example, the CD bands in the region 260–290 nm are suppressed; at 0.1 mg/ml., the same bands reappear with

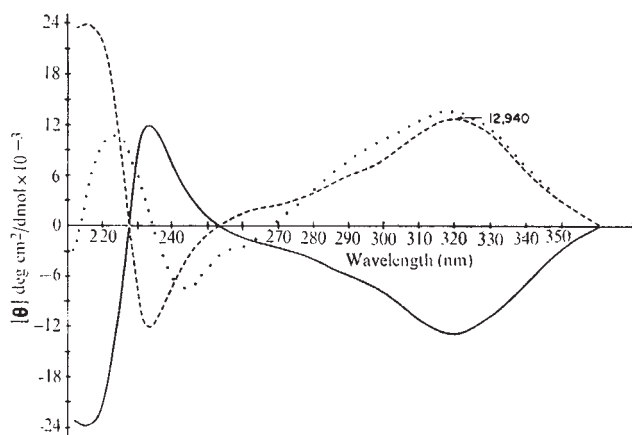


Fig. 1 The circular dichroism of l-LSD (—), d-LSD (---) and d-2-bromo-LSD (···). CD was measured on a Cary 60 spectropolarimeter with circular dichroism attachment, standardized with an aqueous solution of d-10-camphorsulphonic acid (K and K Laboratories, Plainview, New York), giving $E_L - E_R = 2.20 \pm 0.05$ at 290 nm. Solutions at pH 7.0 and 25° C in 0.02 M K_2HPO_4 gave the same spectra with both 0.01 M Tris (hydroxymethyl)aminomethane and 0.15 M NaCl. d-LSD, l-LSD and d-2-bromo-LSD are supplied in an L(+) tartrate buffer at 0.12 mM. Measurements were made at four different concentrations of LSD (maximum 0.01 mg/ml.) to verify conformity with Beer's law (see text). The LSD : tartrate ratio is constant for all of these studies^{2,6}. Over the spectral region 215–370 nm L(+) tartrate does not contribute asymmetrically to the optical activity of d-LSD and l-LSD in the conditions studied. In all studies reported here, the l-LSD used was found to be the optical mirror of the d-LSD, as shown in the figure.

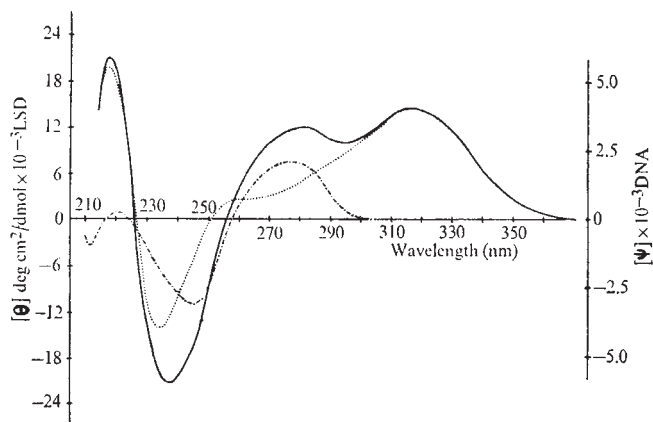


Fig. 2 The circular dichroism spectra of d-LSD (···), DNA (---) and their combination (—). The combination is a simple summation of the component spectra. The summed curves are the same in 10–50 mM Tris, 20–50 mM K_2HPO_4 and 100–150 mM NaCl at pH 6.5, 7.0 and 8.0. These are the conditions used by Wagner. DNA solutions were measured at concentrations of 20, 30, 60, 75, and 120 μ g/ml.; the spectra with added LSD were always the sum of the component spectra. These particular curves were copied from spectra measured on a DNA solution at 20 μ g/ml. d-LSD at 3.3×10^{-5} M. Spectra were measured at room temperature. Calf thymus DNA was purchased from Sigma Chemical Co., St Louis, Mo. Other DNA was supplied by Professors Antero So and Sheldon Greer whose DNA (*B. subtilis*) was shown, apart from physical measurements, to possess full biological activity; the biological activity and integrity of the DNA were determined by assaying the capacity of low concentrations of DNA to transform two genetic markers (*his* 31 and *ind* 168) that are linked to the extent of 65%.

inversion of the 220 nm band. These variations have not been explained but it is unlikely that at such high concentrations they are biologically relevant. As a consequence of this unexplained behaviour of LSD, we restricted our *in vitro* studies to solutions of DNA and LSD in which the LSD concentrations were less than 0.01 mg/ml., concentrations at which the results were reproducible and at which the Beer's law dependence of their spectra is verifiable.

In previous work on this subject, the purity of the LSD sample with respect to isomeric forms was not determined. Some have used a racemic mixture (Wagner, private communication). To ensure that our comparisons between d-LSD and l-LSD are valid, solutions were used which gave mirror-image CD spectra of the particular isomer that was used in each case (Fig. 1). Solutions of each isomer are then pure or at worst slightly cross-contaminated to the same degree by the other enantiomer, because they also give characteristic rotations at the sodium D line.

Studying the circular dichroism of DNA in the presence of d-LSD (the psychotomimetic congener) we found no significant changes; the changes were less than 1% of those reported by Wagner² in the same conditions; the interaction of d-LSD and DNA, shown by the fluorescence quenching experiments of Yehling and Sterglanz, did not cause any conformational change in either the d-LSD or DNA. In the spectral region 400–200 nm, the circular dichroism of the d-LSD-DNA complex results simply from a combination of the individual d-LSD and DNA spectra (Fig. 2). The calculated LSD + DNA and observed spectra are identical. The DNA was considered to be "native" when they gave CD spectra of native DNA, as defined by Brahms and Mommaerts⁸. The absence of any new optically active contribution to the DNA circular dichroism, when d-LSD was present in the DNA solution, was verified in three different solutions, 0.15 M NaCl, 0.01 M Tris and 0.02 M K_2HPO_4 , each at pH 6.5, 7.0 and 8.0. Additional annealing of the DNA to close the small open stranded regions and to correct mismatched base-pairing by warming to 50, 60 or 67° C and slow cooling produced no changes in the ability of

d-LSD to "intercalate" with DNA, that is, it produced no changes in optical activity other than those associated with the LSD and DNA themselves. This was true whether the DNA was heated in the presence or absence of any of the LSD forms. Furthermore, DNA from *Bacillus subtilis*, *Escherichia coli*, bovine spleen, sheep bone marrow and phage T4 gave the same negative results, showing the absence of any interaction for a wide range of (A + T)/(C + G) ratios.

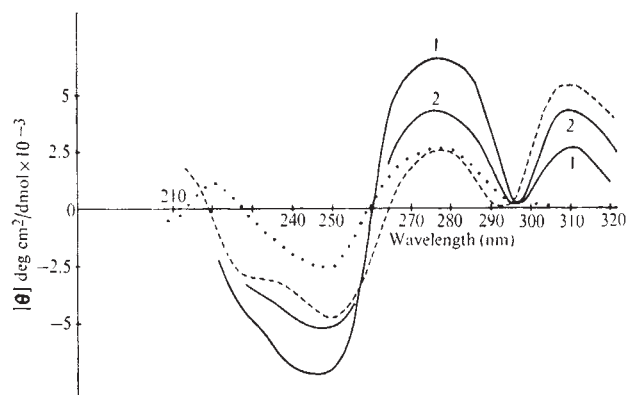


Fig. 3 The CD spectra of ethidium bromide-DNA mixtures. . . . Curve represents pure calf thymus DNA. The differences in intensities between curves 1, 2 and the broken line relate to the age of the DNA solution. Curve 1 describes the freshest DNA solution; curve 2 was obtained from solutions that had been stored at 4° C for a week, and the dashed curve represents a much older solution of DNA, about 4 weeks old. One can obtain the dashed curve on a freshly prepared solution of old DNA which has been stored as a cold dry powder. The dashed curve is closest to the one published by Wagner². Several samples of calf thymus DNA (Sigma) gave even higher ellipticity values at 272 nm with ethidium bromide, and it may be that they were the most "native" of the more than thirty preparations tested, but the curves reported here are the three which proved to be characteristic of three reproducibly different states of DNA. In spite of the variation in ethidium bromide-induced changes, the DNA alone always gave the same CD spectrum. In all cases the concentration of DNA is 30 µg/ml. and the concentration of ethidium bromide is 3.3×10^{-5} M. The more intense spectra were most consistent for solutions in 10 mM Tris, as compared with phosphate and NaCl solutions.

To eliminate artefacts arising from nicking of the DNA during handling as a complicating factor, experiments were done in two ways: in the first, solutions of fresh DNA were gently poured; and in the second, DNA solutions were extruded through hypodermic syringes with small bore needles. But, in spite of the different degrees of shearing, the results of the two were identical; in both cases, the circular dichroism of the LSD-DNA solution was the sum of the individual LSD and DNA spectra.

The fluorescence changes seen by Yielding and Sterglanz on the binding of LSD to DNA indicated to them that the sort of interaction which occurs is a "general property of this group of drugs", and that the "binding ratio was one drug molecule per base moiety in the DNA chain". It would seem, therefore, that while the drugs of this group may interact with DNA in such a way that their fluorescence emission is suppressed, circular dichroism indicates no conformational change, certainly none similar to that observed in DNA in the presence of the trypanocidal dyes, ethidium bromide or isometamidium chloride. In this respect we are in agreement with Yielding and Sterglanz: the behaviour of dn, ln and d-2-bromo-LSD in the presence of DNA is the same, regardless of the form of LSD.

The CD of ethidium bromide-DNA combinations is shown in Fig. 3. Since ethidium bromide is a good example of the planar compounds believed to intercalate with DNA⁹⁻¹¹, its effect on DNA circular dichroism serves as a useful basis for

comparison in assessing the possible effects of LSD. We found, for example, that the character of the ethidium bromide-induced optical activity is influenced considerably by the age of the DNA solution (Fig. 3).

There is no similarity in behaviour between the LSD-DNA solutions (Fig. 2) and the ethidium bromide-DNA complexes (Fig. 3) and it is unlikely therefore that LSD forms this type of complex with DNA. For example, a significant reciprocal behaviour in ellipticity was found (Fig. 3) in the ethidium bromide-DNA interaction between the positive band at 272 nm, which arises from ethidium bromide. It was also observed by Aktipis and Martz⁹⁻¹¹ and it is in accord with Tinoco's¹² general theory of optical activity. It is evidence for a strong interaction between ethidium bromide and DNA which in no way resembles that between DNA and LSD in any of its forms.

We have also studied the CD spectra of combinations of LSD forms with whole tRNA and pure tryptophanyl-tRNA_{trp} of *E. coli*. No additional optical activity was generated beyond the contributions of tRNA and LSD measured singly, and neither d-LSD nor l-LSD affected the capacity of tRNA_{trp} to charge tryptophan.

Recently, it was reported¹³ that LSD does not damage chromosomes of *E. coli* except at very high concentrations and high ultraviolet light intensity. Unfortunately, the isometric purity of the LSD used in this work was not determined and a maleate salt of LSD was used whereas others have generally used an L(+)tartrate salt. We emphasize the importance of measuring the relative concentrations of the dn and ln forms and of a uniformity in the preparations used. The conflicting results of studies of LSD-induced chromosomal damage¹⁴ may be largely attributable to differences in isomer concentrations and buffering agents in the LSD preparations. Furthermore, some early LSD-DNA spectra seem to be inaccurate, and do not provide a firm basis for consideration of the mode of action of LSD.

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LSD-25 Does Not Intercalate in DNA

APART from its hallucinogenic effect, lysergic acid diethylamide (LSD-25) is believed to cause chromosome abnormalities¹⁻³, which have been attributed to a direct interaction of the LSD-25 and DNA⁴. The apparent resemblance of the circular dichroism spectra of the DNA-ethidium-bromide complex and mixtures of LSD-25 and DNA has led to the suggestion that LSD-25 might be intercalated in the DNA⁵.

A more specific and sensitive method for studying intercalation is provided by the interaction of drugs with twisted, circular, covalently closed duplex DNA⁶⁻⁸. By intercalating molecules into the DNA the duplex is unwound and, because the total number of duplex turns and supertwists is a topological invariant, this leads to the unwinding of the supertwists and therefore to the conversion of twisted circles into open circles⁹. This change in form can be followed by sedimentation analysis, or, more conveniently, in a viscometer¹⁵. We have used this method to verify Wagner's suggestion⁵ that LSD-25 intercalates.

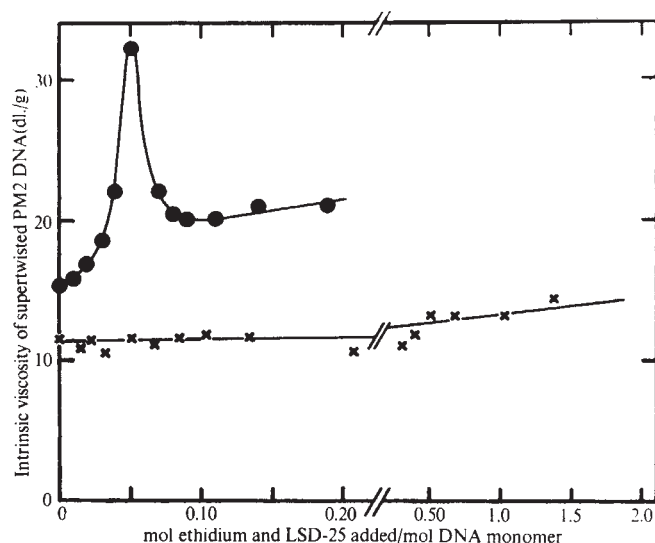


Fig. 1 The intrinsic viscosities of the DNA from phage PM2 as a function of the ethidium (●) and the LSD-25 (×) concentration present. Conditions were: temperature 20° C; DNA concentration, 27 $\mu\text{g ml}^{-1}$ of 15 mM NaCl, 1.5 mM sodium citrate, pH 7.0 (●), and 20.3 $\mu\text{g ml}^{-1}$ of 10 mM sodium phosphate, pH 7.0 (×). The intrinsic viscosities were determined in a rotating cylinder viscometer (Kränich GmbH, Göttingen) according to Zimm and Crothers¹⁰. Ethidium was a gift from Boots, and LSD-25 was purchased from Sandoz.

Fig. 1 shows the results of an experiment in which we compared the effect of LSD-25 and the intercalating dye ethidium on the closed circular duplex DNA of bacteriophage PM2 (refs. 11 and 12). Whereas the results with ethidium were as expected, no interaction between DNA and LSD-25 was detectable, in the range of concentrations in which strong interaction was found by others^{4,5}.

We therefore repeated previous experiments, in which interaction between LSD-25 and DNA was detected spectrophotometrically. In contrast to the results of Yelding and Sterglanz⁴, the spectra of different mixtures of LSD-25 and DNA between 220 and 600 nm were the exact summations of the two components separately; in contrast to Wagner's results⁵, we also found complete additivity for the circular dichroism spectra.

These results indicated that either LSD-25 does not interact directly with DNA, or that our LSD sample contained no LSD-25. The latter alternative was excluded by infrared spectroscopy and NMR studies. We therefore conclude that chromosome damage in the presence of LSD-25 is not a consequence of the intercalation of LSD-25 into DNA.

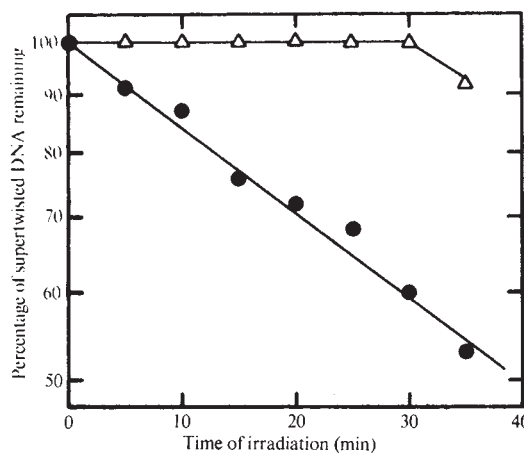


Fig. 2 Breakage of the super-twisted form of PM2 DNA by ultraviolet irradiation in the presence of LSD-25. Samples were irradiated with (●) and without LSD-25 (Δ) with a germicidal lamp. The radiation below 300 nm was cut off by a glass filter. Conditions were: DNA concentration, 63.5 $\mu\text{g ml}^{-1}$; LSD-25 concentration, 310 μM ; medium 15 mM NaCl + 1.5 mM sodium citrate, pH 7.0. The conversion of the super-twisted PM2 DNA was followed by band sedimentation of small samples through neutral 3 M CsCl in an MSE analytical ultracentrifuge. The percentage of super-twisted DNA was estimated from scannings at 260 nm, using a Dupont 310 curve resolver.

During these experiments, we noted that irradiation of DNA with ultraviolet light in the presence of LSD-25 led to phosphodiester bond breakage, even if most of the radiation below 300 nm was cut off with a glass filter. This phenomenon was illustrated by the experiment in which phosphodiester bond breakage in DNA was measured by the conversion of the closed circular duplex form of PM2 DNA to slower sedimenting material (Fig. 2). Electron microscopy confirmed that the irradiation led to the formation of open circles rather than linear molecules. There was no conversion when DNA was irradiated in the absence of LSD-25 or when DNA-LSD mixtures were kept in the dark. Although the conditions used in this experiment were very different from those prevailing *in vivo*, it should be realized that the molecular weight of PM2 DNA is only 6×10^6 , whereas a human diploid nucleus contains 4×10^{12} daltons of DNA¹³. LSD-25-dependent photo-destruction of DNA could therefore contribute to chromosome damage in tissue culture experiments and possibly *in vivo*, and photo-sensibilization of an *Escherichia coli* mutant, deficient in repair, by high concentrations of LSD-25 has been reported¹⁴.

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Induced Optical Activity of 2,4-Dinitrophenyl-lysine Specifically Bound to Mouse MOPC-315 Myeloma Protein

THE optical activity generated by the interaction of small ligands with proteins, visible as extrinsic optical rotatory dispersion (ORD) Cotton effects or circular dichroism (CD) bands, has been used extensively to study the binding of ligands to non-antibody proteins^{1,2}. We describe here the use of circular dichroism to monitor the binding of DNP-lysine to a mouse IgA myeloma protein with high affinity for dinitrophenylated haptens³⁻⁵.

The MOPC-315 mouse tumour line of Potter and Lieberman⁶ was maintained in 5-8 month old BALB/c mice, and the IgA myeloma protein was purified from ascitic fluid by affinity chromatography⁵. DNP-lysine (ϵ -N-2,4-dinitrophenyl-L-lysine)-'Sepharose' was prepared by the method of Porath, Axen and Ernback⁷. We washed a 10 ml. DNP-'Sepharose' column with 200 ml. of 0.15 M NaCl-50 mM sodium borate buffer (pH 8.4), 10 ml. of 1% (w/v) bovine serum albumin, 200 ml. of 1 M acetic acid and 200 ml. of borate-buffered saline immediately before use. MOPC-315 ascitic fluid was dialysed against borate-buffered saline, and the protein was reduced at 25° C with 10 mM dithiothreitol for 1 h, and alkylated for 30 min at 4° C with a 10% molar excess of iodoacetamide. The reduced and alkylated protein was dialysed against borate-buffered saline and applied to a DNP-'Sepharose' column, which was washed with the same solvent, and the MOPC-315 myeloma protein was eluted with 1 M acetic acid. The recovered protein was immediately dialysed against borate-buffered saline (pH 8.4) at 4° C, and insoluble materials were removed by centrifugation. Examination of the purified protein by immunoelectrophoresis with rabbit anti-mouse serum and anti-MOPC-315 protein sera yielded only a single component.

Circular dichroism was measured at 25° C with a JASCO ORD/UV-5 spectropolarimeter equipped with a CD attachment, and standardized with d-camphor-10-sulphonic acid. Cell path lengths of from 1.0 cm to 0.1 mm, and protein concentrations between 0.8 and 4×10^{-3} g per 100 ml., were used to determine the CD spectrum (600 to 200 nm) of the MOPC-315 protein. The data are given in molar ellipticities, $[\theta]_\lambda$, in degrees cm² dmol⁻¹, where $[\theta]_\lambda = \frac{3,300}{lc} (A_L - A_R)_\lambda$, and l = the path length (cm), c = the molar concentration and $(A_L - A_R)_\lambda$ is the observed difference in absorption between left and right circularly polarized light at wavelength λ . The average residue weight, M_0 , taken as 110 (rather than the molecular weight) for the MOPC-315 protein was used to obtain the protein $[\theta]_\lambda$ values. We determined optical density difference spectra with a JASCO ORD/UV-5 spectrophotometer. The quenching of MOPC-315 protein fluorescence by DNP-lysine (Sigma)³ was measured with a Zeiss double M4 QIII monochromator spectrofluorometer with a Farnell stabilized EHT power supply and a Keithley 610 B electrometer, in a thermostatically controlled cell compartment at 25° C. The data were corrected for dilu-

tion and internal quenching as before⁸. Protein concentrations were determined from the optical density at 280 nm (corrected for light scattering), measured with a Zeiss PMQII spectrophotometer, with an extinction coefficient, $E_{1\text{ cm}}^{1\text{ mg mL}^{-1}}$, of 1.44 for the MOPC-315 IgA protein^{3,5}. The molar extinction coefficient, ϵ , at 362 nm for DNP-lysine was taken as 1.75×10^4 (ref. 5).

The CD spectrum of the MOPC-315 protein is shown in Fig. 1A. No circular dichroism was evident in the protein spectrum from 600 to 315 nm. DNP-lysine (up to 1.3×10^{-4} M) in free solution, and in the presence of immunoglobulins lacking DNP-specificity, also had no optical activity in this wavelength range (Fig. 1B). In contrast, binding of the DNP-lysine ligand by the MOPC-315 protein generated distinct circular dichroism bands (Fig. 1B). A broad positive band centred near 410-420 nm, a sharper positive band at 380 nm and a broad negative band extending from 360 nm to 270 nm and centred near 295 ± 15 nm were observed in the CD spectrum of the MOPC-315-DNP-lysine complex (Fig. 1A and B). The absorption spectra of DNP-lysine in free solution, and DNP-lysine specifically bound to the MOPC-315 protein, are also recorded in Fig. 1B. The redshift and hypochromicity³ were evident after complexing the ligand with the myeloma protein, the absorption maximum being shifted from 362 nm for free DNP-lysine ($\epsilon = 1.75 \times 10^4$) to 368 nm for bound DNP-lysine ($\epsilon = 1.52 \times 10^4$).

Hapten-binding curves were constructed by serially adding DNP-lysine (with a Hamilton microsyringe) to a constant amount of MOPC-315 protein, and measuring the circular dichroism at 325 nm and 380 nm. The $(A_L - A_R)_{325\text{ nm}}$ data (uncorrected for dilution) have been plotted as a function of the total DNP-lysine concentration (Fig. 1C). The mean value for $(A_L - A_R)_{325\text{ nm}}$ at saturation (that is $(A_L - A_R)_{\text{max}}$ at DNP-lysine concentrations greater than 8×10^{-5} M), corrected for dilution, was 5.32×10^{-4} . The $(A_L - A_R)_{325\text{ nm}}$ values were divided by the total DNP-lysine concentration to give the apparent value of $[\theta]_{325\text{ nm}}$, and these values are also plotted in Fig. 1C as a function of the total DNP-lysine concentration. The mean of the maximum values for $[\theta]_{325\text{ nm}}$ (apparent) (total DNP-lysine concentration less than 6×10^{-5} M) gave $[\theta]_{325\text{ nm}}$ as -2.10×10^4 degrees cm² dmol⁻¹. Similarly, $[\theta]_{380\text{ nm}}$ was calculated to be 1.68×10^4 degrees cm² dmol⁻¹. The molar concentration of antibody combining sites was determined from the ratio $((A_L - A_R)_{\text{max}}/[\theta]_{325\text{ nm}})$ to be 1.71 mol of sites per 153,000 g of protein.

The intrinsic association constant is an additional parameter for evaluating $[\theta]_\lambda$. The association constant, K_A , obtained for the MOPC-315 protein from the fluorescence quenching data (Fig. 1D) was 0.6×10^7 mol⁻¹, consistent with the value reported by Eisen, Simms and Potter³. The association constant, $K_A = \frac{r}{(n-r)c'}$, where r is the number of moles of hapten

bound per mol of bivalent antibody, $n=2$, and c' is the free hapten concentration^{3,8}. The value of r at each dilution may be obtained from the observed $(A_L - A_R)$ values, corrected for dilution, as the ratio $(2(A_L - A_R)_{\text{observed}}/(A_L - A_R)_{\text{max}})$. At

$r=1$, $c' = \frac{1}{K_A}$, $c = [(\text{total DNP-lysine concentration}) - c']$ and $[\theta]_\lambda = \frac{3,300}{cl} [(A_L - A_R)_\lambda \text{ at } r=1]$. The values for $[\theta]_{325\text{ nm}}$ and

$[\theta]_{380\text{ nm}}$ calculated by this procedure were -2.13×10^4 and 1.66×10^4 degrees cm² dmol⁻¹, respectively, consistent with the values we obtained from the $[\theta]_\lambda$ (apparent) against total DNP-lysine concentration plots (Fig. 1C).

For lower affinity antibodies ($K_0 \sim 10^5$ mol⁻¹), circular dichroism is an alternative method to determine directly the association constant.

The optical activity of the inherently symmetric DNP-chromophore is generated by the interaction of the DNP group with asymmetrically placed vicinal groups in the antibody combining site of the MOPC-315 protein. The reciprocal relationship of

the positive and negative CD bands, together with the large molar extinction coefficient of the DNP group, suggests that the optical activity of the DNP-lysine-MOPC-315 protein complex results (at least partly) from dynamic coupling of electronic transitions of the DNP group and a protein chromophore (for example, tryptophan or tyrosine) in the antibody combining site^{2,9,10}. Little and Eisen¹¹ have studied the difference spectra resulting from the association of DNP ligands and TNP ligands with antibody, and suggested that a tryptophan residue is present in the combining sites of anti-

DNP and anti-TNP antibodies, from several sources. Circular dichroism presents a further experimental parameter with which to explore this possibility. The magnitude of the rotational strength of reciprocal CD bands depends on the relative orientation of the transition dipole moment vectors of the interacting chromophores^{2,10}. Circular dichroism bands which result principally from vicinal perturbations static in nature (for example, asymmetric distribution of charge on vicinal groups) are also sensitive to the relative orientation of the ligand and the amino-acid residues in the antibody combining site. Circular

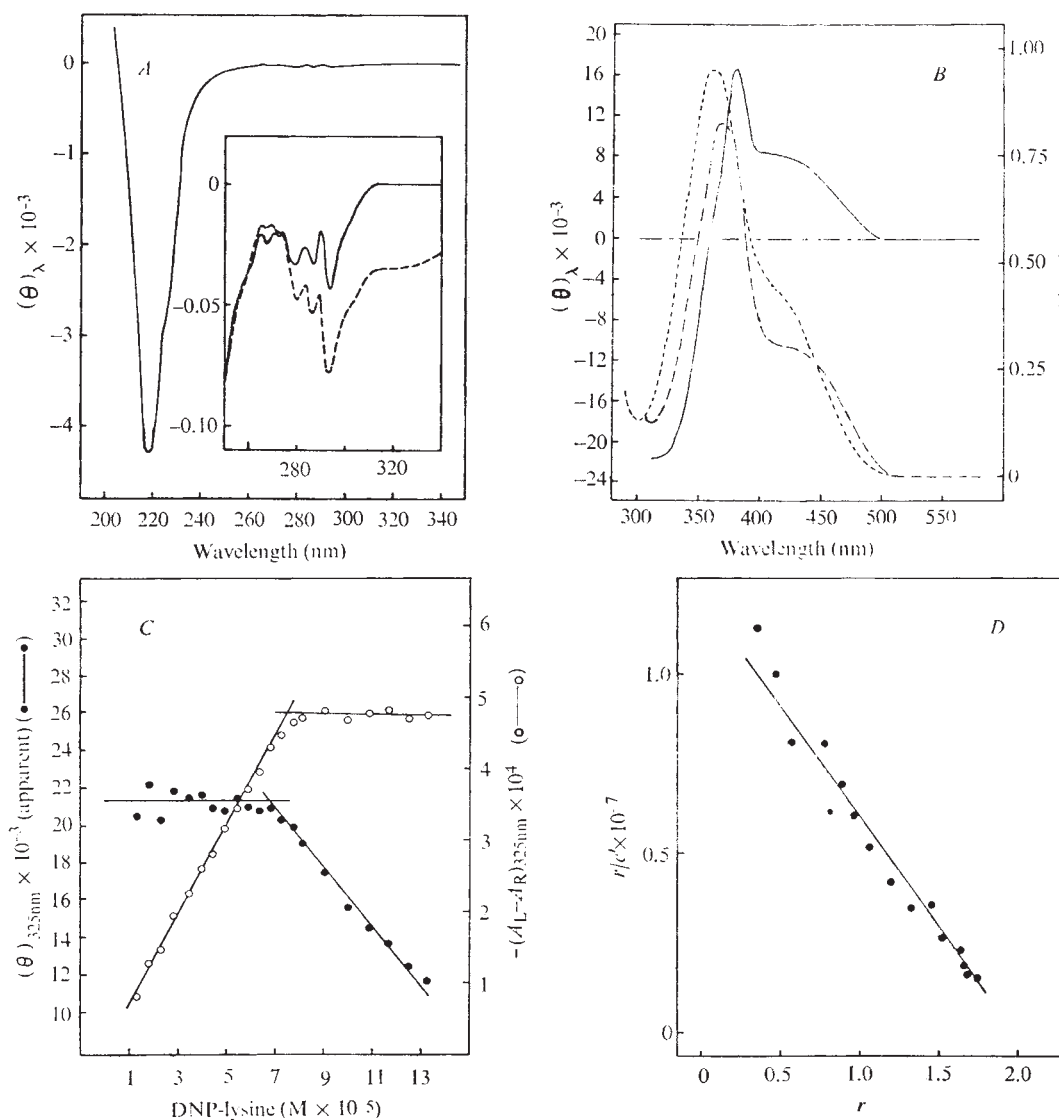


Fig. 1 *A*, Circular dichroism spectrum of MOPC-315 IgA myeloma protein in 0.1 M NaCl-10 mM sodium phosphate buffer (pH 7.2), or 0.1 M NaCl. The inset illustrates the aromatic region with an expanded $(\theta)_\lambda$ scale (—). The circular dichroism spectrum of the DNP-lysine-MOPC-315 protein complex in the protein aromatic amino-acid absorption region is also illustrated in the inset (---). DNP-lysine in free solution showed no circular dichroism from 600 to 220 nm. *B*, Circular dichroism spectrum of ϵ -N-2,4-dinitrophenyl-L-lysine in free solution in 0.15 M NaCl-50 mM sodium borate buffer (pH 8.4) (---), and bound to the MOPC-315 IgA myeloma protein (—). The $(\theta)_\lambda$ values below 315 nm have been corrected for the CD contribution from the protein alone. The absorption spectrum of DNP-lysine (---) and an equal molar concentration of DNP-lysine bound to the MOPC-315 protein (—) are also shown. The same concentration of MOPC-315 protein without DNP-lysine was used as a reference to obtain the difference spectrum of DNP-lysine bound to protein. The DNP-lysine-MOPC-315 difference spectrum was examined at $r=1.2$ where more than 99% of the total DNP-lysine present was specifically bound to protein. *C*, Hapten binding curves constructed by serially adding 10–20 μ l. of 1.07 mM DNP-lysine to 14.8 mg of MOPC-315 protein in 2.0 ml. of 0.15 M NaCl-50 mM sodium borate buffer (pH 8.4). The $(A_L - A_R)_{325\text{nm}}$ data, uncorrected for dilution, have been plotted as a function of the total DNP-lysine concentration (○). The values of $(\theta)_{325\text{nm}}$ (apparent) = $\frac{3,300}{1} [(A_L - A_R)/(\text{total DNP-lysine concentration})]$

have also been plotted as a function of the DNP-lysine concentration (●). *D*, DNP-lysine hapten binding data for the MOPC-315 protein of *C*, obtained by fluorescence quenching. Data are plotted in terms of r and c' , where r is the number of moles of hapten bound per mol of bivalent antibody, and c' is the free DNP-lysine concentration. The molar concentration of antibody combining sites ($n=2$) was determined from the CD binding data (see text).

dichroism, therefore, is a powerful method for the study and comparison of the fine structure of the antibody combining site.

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Localization of A Antigen Sites on Human Erythrocyte Ghosts

DETAILED immunochemical knowledge of the heterosaccharide antigen determinants of the ABO blood groups has been gained from the study of glycoproteins of mammalian secretions¹⁻³. Less is known about the chemical composition and spatial distribution of the ABO antigen determinants in erythrocyte membranes. Because the erythrocyte envelope appears as a uniform unit membrane after positive staining⁴, standard electron microscopy has failed to relate the ABO antigen sites to any discrete membrane structure⁵.

We have demonstrated a new method for the study of the topochemistry of membrane surfaces⁶ which utilizes freeze-etching, a technique involving fracture, which splits the membrane and exposes its inner, hydrophobic matrix^{6,7} (Fig. 1, left), followed by etching, which exposes the membrane surface by lowering the ice table^{6,8} (Fig. 1, right).

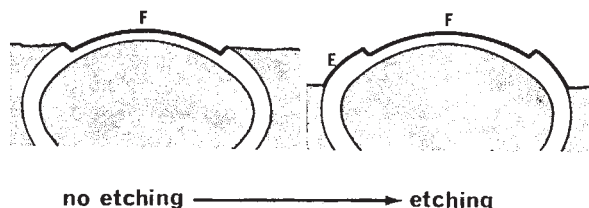


Fig. 1 Faces exposed by freeze-etching red blood cell ghosts: fracture splits the membrane (fracture face, F); subsequent etching lowers the ice table and exposes the membrane surface (etched surface, E). Because the membrane is split, there is a step or ridge between the fracture face and the etched surface.

In the erythrocyte membrane, the hydrophobic matrix exposed by the fracture process is structurally differentiated into smooth areas and particles^{9,10}. The particles average

85 Å in diameter (uncorrected for shadow thickness) and seem to protrude to the membrane surface⁹. Their chemical nature is unknown, although indirect evidence suggests that they are proteins^{9,11-14}. We show here that the A antigen sites of the human erythrocyte ghost are associated with these membrane particles.

Human erythrocyte ghosts from blood group A₁, and controls from blood group O, were prepared¹⁵ from fresh whole blood drawn in acid citrate dextrose buffer. Immune anti-A (Hyland Division, Travenol Laboratories, Los Angeles) was fractionated by chromatography on DEAE cellulose, and the IgG fractions (eluted with 0.01 M phosphate buffer, pH 8.0) were pooled and concentrated by ultrafiltration. The A antigen sites were labelled indirectly with ferritin conjugated to the IgG fraction of rabbit anti-human IgG¹⁶. A₁ or O ghosts (50 µl. of packed ghosts plus 50 µl. of 20 mM phosphate buffer, pH 7.5) were incubated for 75 min at room temperature, with 5 mg of the anti-A IgG fraction in a total volume of 8 ml. After washing three times with 100 volumes of the buffer, the cells were incubated at room temperature with 0.4 ml. of the conjugate for 150 min. The cells were centrifuged and resuspended in cold 20 mosmolar phosphate buffer containing 1 mM MgCl₂, sedimented, resuspended in a 1 : 10 dilution of the same buffer and then washed three times in distilled water. For freeze-etching, portions of the pellet were mounted on cardboard disks and quenched in chlorodifluoromethane ('Freon 22') cooled by liquid nitrogen. Specimens were fractured, etched for 1 min at -100° C and replicated in a Balzers apparatus¹⁷.

Washing the ghosts in distilled water caused appreciable aggregation of almost all the membrane particles (Fig. 2)⁹. The patterns formed by these particle aggregates helped us to recognize their protrusions on the etched surface and enabled us to judge the location of the ferritin molecules relative to the membrane particles (Fig. 2b and c).

Extensive examination of the replicas showed that the ferritin-markers, and thus the anti-A antibodies, were consistently associated with the particle aggregates on the surfaces of A₁ erythrocyte ghosts (Fig. 2b and c). It could be argued, however, that the A antigen sites were not associated with the membrane particles, but that antigens and particles co-aggregated during washing. We thought this unlikely, because if the antigen sites were not associated with the membrane particles random co-aggregation should produce some particle-free antigen aggregates and, consequently, ferritin labelling on the smooth zones of the ghost membrane. We counted the ferritin molecules which corresponded to zones devoid of particles; in a survey of 121 of those smooth zones, only three ferritin molecules were found (there were 296 ferritin molecules on the remainder of the etched surfaces). Furthermore, in an analysis of the position of 1,500 ferritin molecules, only sixteen were not associated with any particle aggregates. Because no ferritin was observed on group O controls, we concluded that the binding of the ferritin was a specific antigen-antibody reaction which localized the A antigen sites on the membrane particles.

The number of marker molecules was much lower than the current estimates of the number of antigen sites of A₁ cells. Our experimental conditions were not chosen to optimize binding to a substantial proportion of the antigen sites, but to minimize agglutination which would make it impossible to expose the outer membrane surface by etching. Preliminary experiments to observe directly the anti-A antibody suggest that most membrane particles possess A antigen sites. This makes the agreement between the current estimates of the number of A antigen sites in A₁ cells ($8-11 \times 10^5$)¹⁸ and the number of membrane particles in the human erythrocyte membrane ($4,200 \mu\text{m}^{-2}$ or about 6.2×10^5 per erythrocyte)^{9,11} interesting¹⁹, but it may be coincidental.

Because the molecular species containing the A and B antigens in the human erythrocyte membrane are probably glycoprotein²⁰⁻²² and glycolipid^{23,24}, the correspondence between the antigen sites and the membrane particles implies

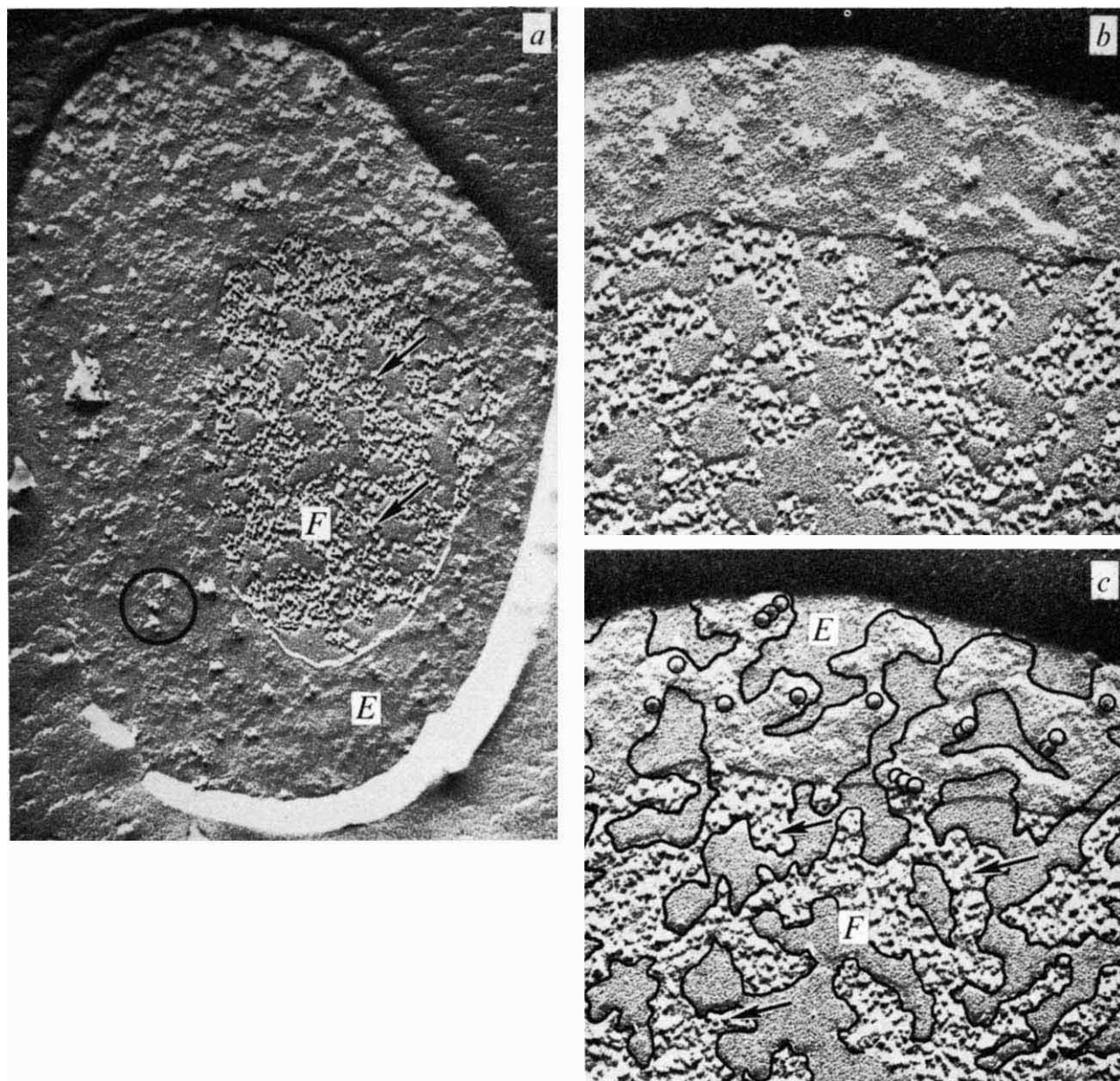


Fig. 2 Freeze-etched A₁ human erythrocyte ghosts labelled with ferritin conjugate indirectly bound to A antigen sites (*a*, 75,000; *b* and *c*, $\times 130,000$). A ridge separates the fracture face (*F*) from the etched surface (*E*). Aggregates of membrane particles (arrows) are visible on the fracture faces and protrude to the etched surfaces where they appear as roughened zones (*b*, outlined and labelled in *c*); the outlines of the roughened zones on the etched surface are continuous with the outlines of the particle aggregates on the fracture face. Ferritin molecules (encircled, *a* and *c*), and therefore A antigen sites, are associated only with the protrusions of the membrane particles on the etched surface.

that the particles contain glycoprotein or glycolipid moieties. Although most of the membrane particles are preferentially associated with the inner half of the membrane^{9,11}, they must protrude to the outer surface where they carry the heterosaccharide ABO determinant. The particles probably have other functions; they seem to traverse the entire membrane, thus providing a structural pathway which may be important in transport phenomena. Extrapolating the present results to the native erythrocyte membrane should be viewed with caution, although preliminary experiments showed that there was no loss of haemagglutination titre in erythrocyte ghosts, either before or after washing in water.

The combination of immunochemical and freeze-etch techniques used here can be extended to study the relationship of membrane particles to other antigen systems, receptor sites, enzymes and transport functions in erythrocyte and other membranes.

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Treatment of Murine Leukaemia with Heterologous Antiserum or Cyclophosphamide

We have compared chemotherapy and immunotherapy in AKR mice injected with a syngeneic tumour, strain BW5147 lymphatic leukaemia. Previous investigations disclosed significant therapeutic benefit from isologous or homologous antiserum against mouse leukaemia¹⁻⁴ or with antiserum raised against murine tumour viruses⁵. In contrast, toxicity has been a significant obstacle to the therapeutic use of heterologous antiserum^{6,7}. Reports⁸⁻¹² of successful use of heterologous antiserum, however, suggested that further study was warranted, and an experiment was designed to compare quantitatively the therapeutic activity of antiserum with conventional chemotherapy.

Strain BW5147 leukaemia was invariably lethal for untreated AKR mice. Median survival time (MST) was 10.0 ± 0.6 days after injection of 10^6 cells intraperitoneally in thirty-two passages utilizing 200 mice. Dispersed cell suspensions were prepared as described previously¹³.

Cyclophosphamide ('Cytoxan') was selected for chemotherapy because of previous studies with L1210 leukaemia, in which a 10^{-3} (99.9%) reduction in tumour was achieved with 100 mg/kg¹⁴. Toxicity of cyclophosphamide in AKR mice was similar to toxicity in other mice¹⁵, and LD₅₀ in fifty AKR mice was 375 mg/kg.

Antitumour serum (ATuS) and antilymphocyte serum (ALS) were prepared by intravenous injections of tumour cells or normal AKR spleen and thymus cells in New Zealand rabbits. Serum was decomplexed and AKR red blood cell (RBC) absorption was performed until haemagglutination titre was negative. In four preliminary experiments, tumour-injected mice treated with RBC-absorbed ATuS had an increase in MST of 42-89% (ref. 16). MST was not prolonged by ALS or normal rabbit serum.

The effects of cyclophosphamide and RBC-absorbed ATuS were compared after injection of 10^6 BW5147 cells intraperi-

toneally. Single cyclophosphamide doses of 20, 40, 60, 80, 120 or 160 mg/kg were given. ATuS was administered in doses of 3.0 ml. once, 1.5 ml./day for 3 days, or 0.25 ml./day until death. Untreated control mice were injected intraperitoneally with serial ten-fold dilutions of tumour cells from 10^2 to 10^7 ; MST with 10^6 cells was 9.50 days.

Increased survival was observed in tumour-injected mice which were treated with either cyclophosphamide or ATuS, and was proportional to the total dose of drug or serum administered. The most effective ATuS regimen (0.25 ml./day) prolonged MST 53%, to 14.50 days. This was similar to the effect of 60 mg/kg of cyclophosphamide, or a 99.99% reduction in tumour inoculum, to 10^2 cells (Fig. 1).

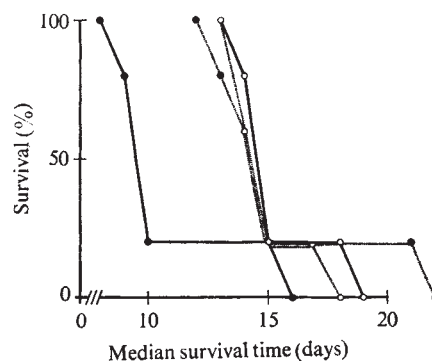


Fig. 1 Effect of rabbit antiserum, cyclophosphamide, or tumour dose reduction on median survival time.

	Tumour	Rx	MST	% prolonged
●—●	10^6	0	9.50	0
●---●	10^2	0	14.25	50
○—○	10^6	ATuS	14.50	53
○---○	10^6	Cy60	14.25	50

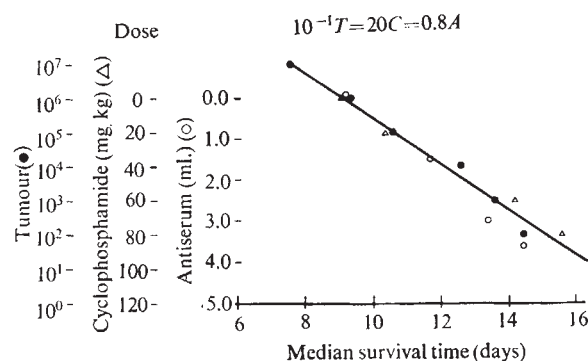


Fig. 2 Therapeutic equivalence of cyclophosphamide, rabbit antiserum and inoculum reduction (ATuS 5-69).

The dose related effects on survival are simultaneously displayed in Fig. 2, and are approximated as follows:

$$10^{-1} T = 20 C = 0.83 A$$

where T is the tumour dose (cells); C is the cyclophosphamide dose (mg/kg); A is the antiserum dose (total, ml.).

This experiment provides direct evidence for the hypothesis^{17,18} that the effect of heterologous antiserum may compare favourably with the effect of chemotherapy in murine leukaemia. When produced and absorbed by methods similar

to those used for ALS^{19,20}, heterologous antiserum seems to be of significant therapeutic benefit in passive immunotherapy of murine leukaemia.

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Development of a Specific Anti-leukaemic Serum for the Treatment of Leukaemia in Clinics

LEUKAEMIC cells are antigenically indistinguishable from their normal counterparts and antibodies formed against them are therefore likely to exhibit cross-reactivity with normal cells. There is, however, a difference in the net surface charge¹—the charge which regulates the ability of the antibodies to

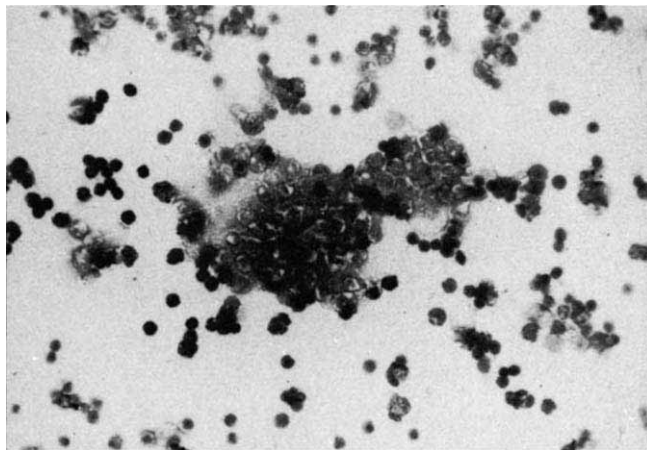


Fig. 2 Leukaemic cells treated with specific anti-leukaemic serum. Note the clumping and disintegration of the clumped up leukaemic cells.

approach the target cells—which could be exploited to prepare antibodies specific to leukaemic cells. To this end, the net surface charge on the white blood cells of normal O group people was suitably altered by tagging the cells with chemicals such as 1-fluoro-2,4-dinitrobenzene (FDNB). These cells were then used as antigens for producing antibodies against human leukaemic cells in rabbits and horses. Here we give a brief account of the production of purified serum specific for leukaemic cells using immunized horse. A preliminary report on the production of specific anti-leukaemic serum from immunized rabbit has already been published².

Heparin, citrate or oxalate was added as anticoagulant to 300 ml. of blood from normal O group healthy people. Red blood cells were allowed to settle overnight with the help of dextran (5 ml. of 6% dextran to 100 ml. of blood). Supernatant and buffy coat were centrifuged to isolate the white blood cells. These were then alternately washed with normal saline and centrifuged at 600–800 r.p.m. at least five times to free the cells from plasma and anticoagulants. The few contaminating red blood cells were destroyed by a 30 s selective hypotonic shock^{3,4} and the white blood cells were washed free of haemoglobin and stroma. This process was repeated until the white blood cells (WBCs) were completely uncontaminated. The viability of the WBCs was checked by the trypan blue dye-exclusion test of Ling⁵. They were then resuspended in normal sterile saline.

Saline suspension (1 ml.) containing 10^5 WBCs was mixed with 1 ml. of FDNB solution containing 10^9 molecules. The pH was adjusted to 7.2 and the mixture was shaken for 10 to 15 min at room temperature (22°–25° C). Untreated FDNB, if any, was removed from tagged cells by repeated washing and centrifuging (600–800 r.p.m.) with ten volumes of saline. FDNB tagged cells were resuspended in isotonic saline (10^7 cells/ml.). The tagged cells were again checked for viability by the dye-exclusion test.

Cell suspension (10 ml.) was injected subcutaneously to a horse each week for 22 weeks. The horse was then bled and the serum was prepared. The serum was heat inactivated and absorbed with pooled A, B and O human RBCs; the globulins were separated by ammonium sulphate and isoelectric precipitation⁶. The purified serum was tested for agglutination with leukaemic cells and normal WBCs (Figs. 1 and 2). Heparin was used as an anticoagulant⁷ in the collection of blood samples. Non-specific aggregation of WBCs was prevented by minimizing the exposure of the WBCs to the anticoagulant (heparin) and by centrifugation at low speed (600–800 r.p.m.)⁵ during the separation and washing of the cells. Titres were evaluated by the usual double dilution technique. Serial dilutions of the antisera were made with normal saline. Samples of 0.03 ml. containing 10^5 cells and 0.03 ml. of anti-sera at various dilutions were mixed in siliconized micro-

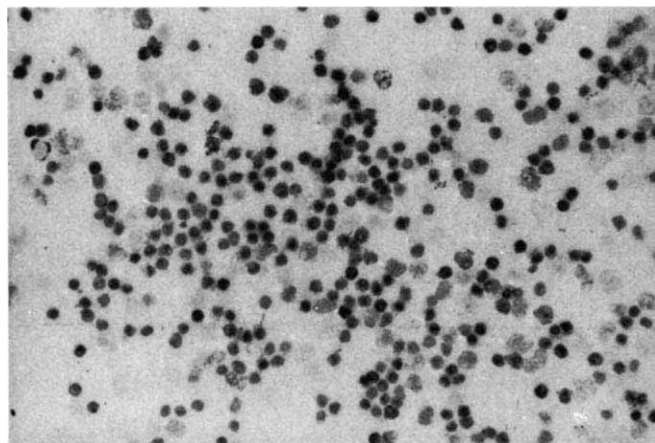


Fig. 1 Normal human WBCs treated with specific anti-leukaemic serum. No agglutination was seen.

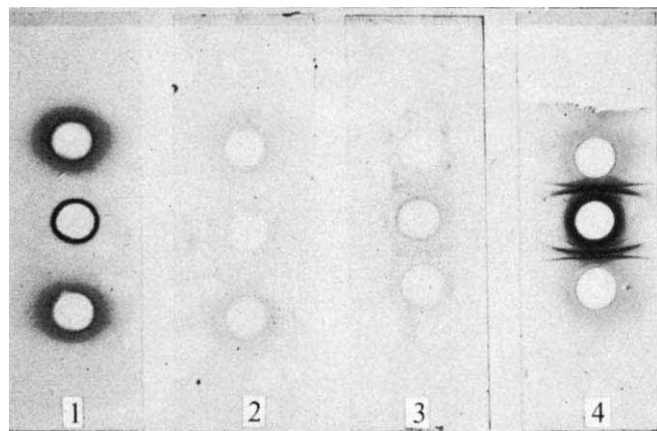


Fig. 3 Specificity of the anti-leukaemic serum demonstrated by the Ouchterlony gel diffusion method. Precipitation lines are obtained with leukaemic cells only. WBCs from normal subjects as well as plasma from normal or leukaemic persons did not reveal any cross-reactivity. Central well, anti-leukaemic serum. Top and bottom wells: 1, human plasma from normal persons; 2, human plasma from leukaemic patients; 3, membranous extracts of white blood cells from normal persons; 4, membranous extract of leukaemic cells. The extracts in wells 3 and 4 were from cells lysed by osmotic shock and the crude membranous material was extracted with mild alkaline solution (pH 7.4).

Table 1 Agglutination Titre with Purified Anti-leukaemic Serum against Leukaemic Cells separated from Leukaemic Patients

Patient No.	Blood group	Agglutination titre	Clinical diagnosis
Myeloid leukaemia cases:			
1	O	4,096	CML
2	A	8,192	CML
3	O	4,096	CML
4	B	4,096	CML
5	B	2,048	CML
6	O	2,048	CML
7	O	4,096	CML
8	AB	2,048	CML
9	A	2,048	CML
10	B	4,096	CML
11	O	4,096	AML
Lymphatic leukaemia cases:			
1	B	8,192	CLL
2	B	4,096	ALL
3	O	8,192	CLL
4	O	4,096	ALL
5	B	8,192	CLL

CML, Chronic myeloid leukaemia; AML, acute myeloid leukaemia; CLL, chronic lymphatic leukaemia; ALL, acute lymphatic leukaemia.

Table 2 Agglutination Titre with Purified Anti-leukaemic Serum against White Blood Cells from Normal People

No.	Blood group	Agglutination titre
1	B	0
2	O	0
3	A	0
4	B	0
5	O	4
6	A	0
7	B	0
8	O	0
9	B	0
10	B	0
11	O	4
12	O	0
13	B	2
14	AB	0
15	O	0

precipitin tubes and incubated at 37° C for 1 h. The tubes were centrifuged at 600–800 r.p.m. for 1 min and the degree of agglutination was determined. The maximum dilution of the serum which showed definite clumping (+1) was taken as the titre of the antisera. The specificity of the antiserum as well as the cross-reactivity, if any, with the plasma from normal human subjects and from leukaemic patients were studied by gel diffusion.

The agglutination titres are given in Table 1. The leukaemic cells were from a representative sample of sixteen leukaemic patients, eleven of whom had myeloid leukaemia, the other five having lymphatic leukaemia. Both types produced high titres compared with agglutination titres with normal WBCs (Table 2). The titres varied between 2,048 and 8,192 in both cases. Titres were negligible when normal WBCs were used.

The specificity of the anti-leukaemic serum was confirmed by clear-cut precipitin reactions with the leukaemic cells and by the lack of such precipitin reactions with normal WBCs (Fig. 3). Plasma proteins either from normal or leukaemic persons did not show any cross-reactivity. It is therefore clear that the anti-leukaemic serum is highly specific for leukaemic cells in general. A detailed report of purification, pharmacological studies and clinical trials will be published later.

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Induction of Specific Autoimmune Response to Leukaemic Cells in Human Leukaemia Patient by Chemically Tagged Normal "O" Group White Blood Cells

WHITE blood cells from normal healthy O group people, when suitably tagged with chemicals such as 1-fluoro-2,4-dinitrobenzene (FDNB), can be used as antigens to stimulate the production of antibodies to human leukaemic cells in rabbits and horse^{1,2}. We have shown that chemically tagged normal O group white blood cells can be used successfully to induce a specific autoimmune response to leukaemic cells in human leukaemic patients.

The serum of a 26 year old male patient with acute lymphatic leukaemia who had been treated with 'Endoxan' (100 mg/day), 6-mercaptopurine (150 mg/day) and prednisolone (60 mg/day),

and who was on a maintenance dose of prednisolone (5 mg/day) when the experiment was initiated, was tested for the presence of iso-antibodies against leukaemic cells from other patients. He was then immunized for 7 weeks with chemically tagged O group WBCs as described below.

Blood was periodically withdrawn to check the iso-antibody levels. Because the patient had a history of short remission periods, 6-mercaptopurine (150 mg/day) was administered in addition to the prednisolone during the immunization period. The patient was discharged from the hospital after seven weeks of the immunization schedule and a blood sample was obtained a month later in order to check the antibody levels.

O group WBCs from normal individuals were tagged with 1-fluoro-2,4-dinitrobenzene (FDNB)². A sample (1 ml.) containing 10^7 FDNB-tagged cells was injected subcutaneously into the patient each week. The site of injection was varied. The patient experienced no discomfort or ill effects as a result of injections and the schedule was continued for 7 weeks. Blood (10 ml.) was withdrawn before the immunization schedule began to find out whether there were any inherent iso-antibodies present. This was repeated each week to check the production of iso-antibodies against leukaemic cells. The iso-antibody titre was determined by measuring the degree of agglutination with leukaemic cells from other patients, and also by studying the agar-gel precipitin reaction with leukaemic cells. Both procedures have been described before².

The immunological responses of patients with acute lymphatic leukaemia are believed to be comparatively poor. In

addition, the patient was being given prednisolone, an immunosuppressive drug³, as a maintenance dose. Yet the iso-antibody titre increased to 256 after 4 weeks (Fig. 1). The agar-gel diffusion test also revealed a single, clear precipitin line (Fig. 2). During treatment, when the patient was dosed with 6-mercaptopurine (150 mg/day), another potent immunosuppressive drug³, the iso-antibody titre did not increase but remained at 128. The iso-antibody titre remained at that level even after the immunization schedule had been discontinued. This investigation suggests that it is possible to elicit a specific auto-immune response to leukaemic cells by using non-malignant antigens. Further data on use of active immunization in patients and their clinical response will be published later.

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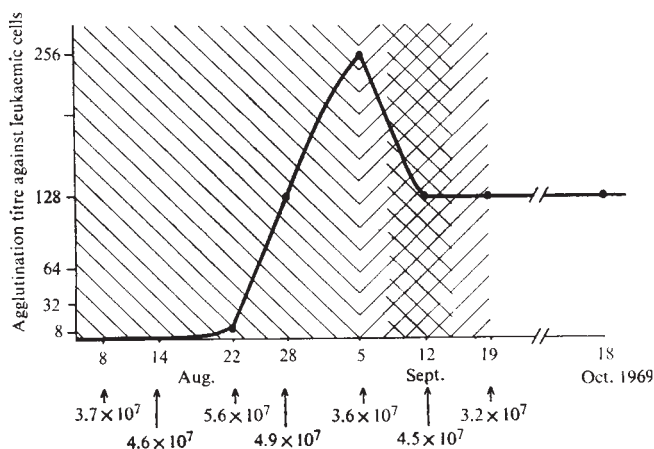


Fig. 1 Development of antibodies specific to leukaemic cells in human acute lymphatic leukaemia patient. Arrows indicate the schedule of immunization. ▨, Prednisolone administration; ▨, 6-mercaptopurine administration.

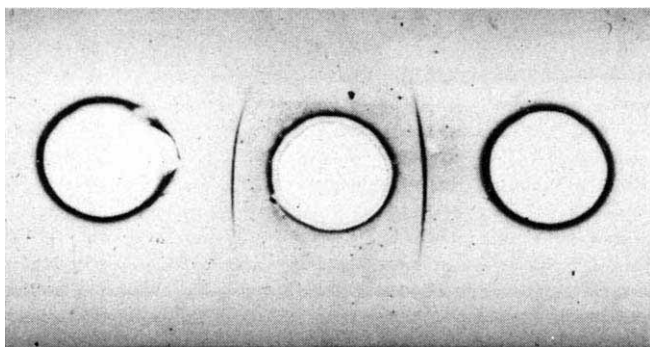


Fig. 2 Demonstration of specific precipitation reaction with the patient's serum against leukaemic cells by Ouchterlony gel diffusion method. Clear-cut precipitin lines were seen on either side of the central well. Central well: patient's serum after four immunizations; side wells: extracts of leukaemic cell membranes.

Synthesis of Nitrosopiperidine from Nitrate and Piperidine in the Gastro-intestinal Tract of the Rat

MANY nitrosamines are carcinogenic to a wide range of animal species¹⁻⁴. The synthesis of nitrosamines from nitrite and secondary amines has been demonstrated *in vitro* with animal and human gastric juice^{5,6} and *in vivo* in animals⁶; nitrosamine synthesis was pH-dependent⁶⁻⁹. The synthesis of the non-carcinogenic diphenylnitrosamine has been reported in the human stomach after oral administration of nitrate and diphenylamine⁷. Nitrosamines can also be synthesized from nitrate and secondary amines *in vitro* at near neutral pH in the presence of enteric enteria¹⁰. We have demonstrated *in vivo* synthesis of nitrosopiperidine from nitrite and piperidine in the stomach and small intestine of the rat¹¹. We report here *in vivo* synthesis of the carcinogenic nitrosopiperidine from nitrate and piperidine in the rat stomach and small intestine, and also with rat gastric contents *in vitro*.

Male Sprague-Dawley rats weighing 200–275 g were used. Methods used for *in vitro* studies with the gastric contents and for *in vivo* studies using the stomach or small intestine have been described before¹¹. The ranges of pH values for stomach and small intestine were 3.4–4.3 and 6.3–6.8, respectively. For *in vivo* studies, solutions of sodium nitrate and piperidine were incubated for 30 min in the isolated intestine and/or in the

stomach of the same rat or of different rats (Table 1). After incubation, intestinal and stomach contents were removed and extracted with dichloromethane as described before¹¹; the solvent extract was dried overnight with sodium sulphate using a mechanical shaker. Nitrosopiperidine in extracts was quantitatively determined by gas-liquid chromatography¹¹. The identity of nitrosopiperidine was confirmed by thin layer chromatography¹² and by additional use of another column of 3% QF-1 (Dow Corning fluoroalkyl silicone) on silanized gas chrom p. Using these techniques, the recovery from saline of a standard sample of pure nitrosopiperidine was 35%.

Table 1 Formation of Nitrosopiperidine from Sodium Nitrate and Piperidine Hydrochloride

Repli- cate *	Reactant con- centrations (mg)		Nitrosopiperidine yield † (µg)			
			In different rats		In the same rat	
	Piper- idine HCl	NaNO ₃	Gastric juice <i>in vitro</i>	Small intest- ine <i>in vivo</i>	Stomach <i>in vivo</i>	Small intest- ine <i>in vivo</i>
1	1,250	25	25	44	54	39
2	1,250	25	27	40	20	30
3	1,250	25	24	41	8	38
4	1,250	25	13	8	2	32

* Each replicate is based on four rats.

† Estimated by gas-liquid chromatography.

Nitrosopiperidine was synthesized from nitrate and piperidine *in vivo* in the stomach and small intestine and from gastric juice *in vitro* (Table 1). Yields of nitrosopiperidine *in vivo* were generally somewhat higher in the stomach than in the small intestine, following incubation in the same or in different rats. Yields *in vitro* in the gastric juice were comparable with *in vivo* yields. These yields of nitrosopiperidine are underestimates in view of the relatively low recoveries of standard samples of nitrosopiperidine. Additionally, no effort was made to correct for nitrosopiperidine possibly absorbed during *in vivo* incubation.

The yields of nitrosopiperidine following *in vitro* and *in vivo* synthesis from nitrite and piperidine¹¹ were greater than for corresponding yields from comparable concentrations of nitrate and piperidine, as reported here. The synthesis of nitrosopiperidine even in small amounts, however, may have cumulative toxic effects, for nitrosopiperidine is a potent carcinogen¹³. Our results are in accord with human data on *in vivo* gastric synthesis of diphenylnitrosamine from nitrate and diphenylamine⁷; bacterial reduction of nitrate and nitrosamine synthesis in these studies was pH-dependent. The synthesis *in vivo* of the carcinogenic nitrosamine nitrosopiperidine from nitrate and piperidine is of practical interest in view of the common dietary occurrence of these precursors. Piperidine is a urinary constituent of normal human subjects, presumably formed by intestinal bacterial decarboxylation from dietary lysine¹⁴. Piperidine may also be ingested by man through some foods and spices; some bread flavouring agents contain piperidine¹⁵. Sodium nitrate and nitrite are used in the United States and elsewhere as preservatives and colour fixatives in cured meats and fish at permissible levels of 500 and 200 p.p.m. respectively¹⁶. Nitrite is also used alone at levels below 10 p.p.m. in smoked and cured tunafish. The use of nitrate or nitrite in certain types of cheese is permitted in some European countries. Nitrates are also widely distributed in many plants. Certain plants such as beets and spinach accumulate nitrates, especially when overfertilized¹⁷ and in conditions of molybdenum deficiency¹⁸⁻²⁰. Further, nitrate levels in drinking water, particularly in certain agricultural areas, may be high enough to induce acute methaemoglobinaemia in infants²¹⁻²⁵. The transplacental transmission of nitrates and the development of methaemoglobinaemia in the fetuses of rats drinking water with high concentrations of nitrates has been recently demonstrated²⁶. The implication of these findings to human

cancer and the propriety of the continued use of nitrate and nitrite as food additives, and of drinking water with a high nitrate content, clearly merits critical consideration.

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Distribution and Concentration of Mercury in Autopsy Specimens of Human Brain

THE dangers of mercury, a pollutant of natural bodies of water and a metal with a strong tendency to accumulate in the nervous tissues, have recently caused considerable concern. We have studied the distribution and concentration of mercury in the brain in order to determine the levels normally present.

Eight autopsy specimens were selected at random for the study. The subjects (Table 1) had lived, at least briefly, in an area where mercurial contamination of the regional inland waters and their fish has been demonstrated, but there was otherwise no known pathological exposure to the metal. The area includes Buffalo, New York, and the adjacent Great Lakes. Six brains were taken from the subjects 6-30 h after death; Nos. 1 and 2 were fixed in formalin before dissection. The tissues were analysed for mercury by neutron activation

Table 1 Content of Mercury in Various Regions of the Human Brain

Subject No.	1	2	3	4	5	6	7	8
Age and sex (yr, M/F)	70M	58M	73M	68F	79M	74M	33M	Full term stillborn M
Area of brain								
Frontal lobe cortex	—	0.25	0.12	0.03	0.11	1.69	0.06	0.04
Cerebellar cortex	0.71	0.72	0.67	0.08	0.48	1.85	0.10	0.04
Medulla	—	—	0.12	0.04	0.18	1.29	0.19	—
Pons	0.43	0.30	0.19	0.05	0.25	1.66	0.05	—
Mid brain	—	0.24	0.11	0.04	0.08	1.20	0.06	—
Corpus striatum	—	0.11	0.09	0.03	0.06	0.61	0.06	—
Thalamus	—	0.16	0.07	0.04	0.11	1.47	0.09	0.04
White matter	0.15	0.13	0.03	0.03	0.04	0.15	0.02	0.05
Angular and supramarginal Gyri	—	0.23	0.07	0.03	0.15	2.00	0.04	0.04

Values are expressed as p.p.m. of wet weight tissue.

analysis (presented by S. K. K. P., C. C. Thomas, jun., and J. A. Söndel at meeting of Amer. Chem. Soc., 1971). After neutron irradiation the tissues were wet ashed in a nitric, sulphuric and perchloric acid mixture with mercury carrier in reflux conditions. A preliminary sulphide precipitation was followed by isolation of the mercury by electrodeposition. The radioactivities from ^{197}Hg and $^{197\text{m}}\text{Hg}$ were measured by gamma-ray spectrometry to determine the levels of the metal in the samples. This method readily allowed the quantitation of mercury in the range 0.01 to 2.0 $\mu\text{g/g}$ of wet tissue with a sample size of 1–3 g. The accuracy was better than 15% at mercury levels of 0.01 p.p.m. and exceeded 5% at concentrations of 2 p.p.m.

The relatively small number of patients precluded the definition of absolute normal values but some valid observations can be made. As observed in Table 1, patient No. 6 is remarkable in that he had very large amounts of mercury in all regions of the brain except in the white matter. This patient had been considered a chronic alcoholic with chronic brain syndrome. He had acquired syphilis in 1929 but it could not be determined whether he had been treated with mercurial preparations.

Generally, the highest concentrations of mercury were found in the cerebellum. This led us to consider whether this structure normally contains relatively high levels of mercury or whether this element is preferentially accumulated at this site. The former assumption would appear tenable, at least to some degree, for in those subjects whose cerebellar values were not maximal, either all the regions of the brain including the cerebellum had very high levels (No. 6) or the cerebellar concentration tended to be relatively prominent (No. 7). Further support for this thesis can be inferred from the data of subject No. 4. The brain of this patient consistently contained the lowest or nearly the lowest concentrations of mercury in all areas studied in comparison with other subjects. The concentration in the cerebellum nevertheless exceeded the other areas in this brain in all instances but one by a factor of at least two. Conversely, the high incidence of clinically demonstrable cerebellar malfunction and gross cellular destruction in this region in patients with documented mercurial poisoning supports the concept of localized preferential accumulation^{1,4}. The significance of the mercurial concentration in the foetal cerebellum (0.04 p.p.m.) could not be readily evaluated because of incomplete sampling due to post-mortem autolysis.

The pons is also of interest since in patients Nos. 2, 3, 4 and 5 the pontine concentration of mercury was exceeded only by that of the cerebellar cortex.

Except for the one brain that had a larger concentration of mercury (No. 6), the sampled areas of the formalin-fixed brains (Nos. 1 and 2) contained a greater amount than the other specimens. An analysis of the fixative was not performed.

The white matter of the hemispheres consistently had the lowest or near lowest levels in all individuals. This low profile suggests a correlation between anatomical structure and accumu-

lation of the metal. The white matter is primarily composed of nerve fibres and is devoid of the cell bodies of nerve cells. Thus, this metal seems to be predisposed to localization in the perykarya of nerve cells rather than in dendritic or axonic processes. The ability of mercury to enter enzymatic reactions as well as its presence in microsomal and mitochondrial fractions of mercury-loaded nervous tissue⁵ are consistent with this concept.

All regions of the brain examined revealed the presence of mercury. Since the observed levels are within the limits of accuracy of the method used, mercury may well be present as a normal constituent of neuronal tissue. Further studies are necessary to confirm this possibility.

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Accelerated Elimination of Methyl Mercury from Animals

WE wish to suggest one principle that may be valuable in the treatment of methyl mercury poisoning—the notion that continuous oral administration of indigestible unabsorbable SH-compounds such as reduced hair powder may accelerate the elimination of methyl mercury from mice.

Organic mercury injected into animals is known to be^{1,2} fairly well excreted into the bile for a considerable time and we have confirmed this result and have studied the chemical nature of the mercury excreted into bile after three rats (weighing about 200 g) were intramuscularly injected with 2 mg of methyl mercuric chloride each day for 5 days. The rats were killed 48 h after the last injection and the contents of the small intestine from three were washed out with 0.95 M phosphate buffer (pH 7.0) and pooled. The total mercury

found in this material was 106.6 μg and about 85% of it was found in the high speed supernatant. The supernatant was used for the following studies. When a portion of it was treated with 1 M HCl for 1 day, extracted with benzene, and run on a gas chromatograph column containing 10% 1,4-butanediol succinate on 'Chromosorb W' and fitted with an electron capture detector, 83% of the mercury was found to be in the form of methyl mercury. It is not clear whether the rest of the mercury is organic or not. When the supernatant was directly extracted with benzene, 23% of the mercury was present as free methyl mercuric chloride. And when the supernatant was dialysed against forty volumes of 0.05 M phosphate buffer (pH 7.0) for 24 h, 80% of the mercury was found to be in the form of dialysable, low molecular derivatives. When 1 g of human hair powder treated with thioglycollate as described later was added to the supernatant, 82% of the mercury was bound to the hair powder and precipitated during 30 min incubation at 37° C. After repeating the experiment and obtaining a very similar result, our conclusion was that most of the mercury excreted in the bile was methyl mercury in the form of its chloride or low molecular weight compounds.

We thought that trapping these forms of mercury and protecting them from reabsorption from the intestine would decrease the accumulation of mercury in the animals. To test this idea we prepared human hair powder reduced with thioglycollate, a substance that has a good methyl mercury binding capacity because of the cysteine SH group and because it is resistant to trypsin digestion. About 20 g of clean human hair was cut into very small pieces with scissors and ground with a strong conical homogenizer in 400 ml. of dilute ammonia (pH 11) containing 9.3 g of thioglycollic acid. After adding another 5 g of thioglycollic acid the mixture was allowed to stand at room temperature for 2 h under nitrogen. The precipitates were collected by centrifugation, washed once with plenty of water and three times with acetone and with ether. The material was dried and stored in an evacuated desiccator. One gramme of this material could bind 60–70 mg of methyl mercuric chloride. It could also recover 80% of 100 μg of methyl mercuric chloride injected into mice. In this experiment, a whole mouse (20 g) was ashed with 20 ml. of concentrated HNO_3 and 20 ml. concentrated H_2SO_4 for 5 h and then reheated for 30 min before addition of 5 g of potassium permanganate. The mercury content of the ash and the recovery were measured by the dithizone method^{3,4}. In a feeding experiment twenty-eight female mice (16–17 g) of the DD strain were poisoned by a single intramuscular injection of 200 μg of methyl mercuric chloride in saline solution and divided into two groups; one was fed on ordinary food (CREA II) and the other was fed the same diet plus 3.3% hair powder for 10 days. In another experiment thirty-four mice were poisoned with 400 μg of methyl mercuric chloride

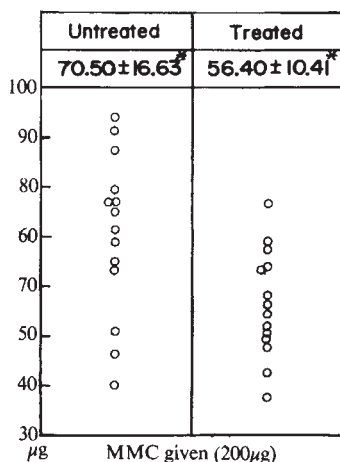


Fig. 1 Total mercury after 10 days. *Mean $\pm$ s.d. The difference was significant ($P < 0.005$).

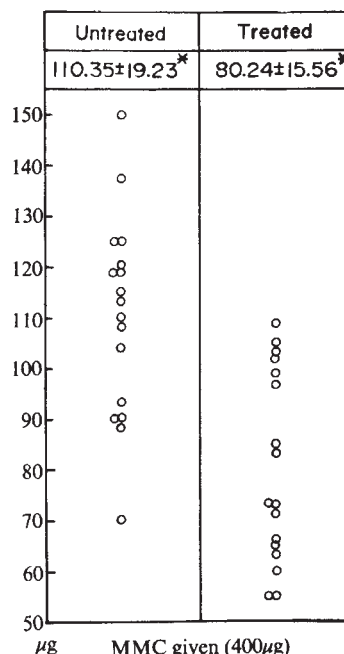


Fig. 2 Total mercury after 20 days. *Mean $\pm$ s.d. The difference was significant ($P < 0.005$).

and the feeding was continued for 20 days. They were then killed. The results of mercury analysis of each animal are shown in Figs. 1 and 2.

The reduced human hair powder reduced the level of the residual methyl mercury in the mice poisoned with methyl mercuric chloride, indicating that methyl mercury excreted into intestine is eliminated by this procedure in the faeces. The results also suggest that reabsorption of methyl mercury excreted into bile is one of the reasons for the prolonged biological half life of methyl mercury in the animal and that any material which has methyl mercury binding capacity and is resistant to digestion and absorption from the intestine could be useful for eliminating methyl mercury from animals. Third, synthetic resins with SH groups may be effective for the same purpose. The procedure may be used in cases of accidental ingestion of organic mercury or it may be used prophylactically when food is consumed that is contaminated with methyl mercury.

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Two-stage Recovery of a Response in *Sepia*

A RESPONSE that has waned as a result of continuous stimulation without reinforcement may reappear when the stimulus is presented on another occasion: habituation, as defined by Thorpe¹, is "relatively permanent" but not complete. The extent of recovery of the response will clearly be a function of the length of time between presentations, but several workers, notably Hinde^{2,3}, have shown that the relation between re-

covery and rest interval is not a simple one. Recovery seems to occur in at least two stages, possibly reflecting the existence of separate underlying physical processes. Some recent experiments with the cuttlefish, described here, have revealed a two-stage recovery with much sharper separation than has been found previously.

Cuttlefish (*Sepia officinalis* L.) feed on prawns, which they capture by a rapid strike with the paired tentacles⁴. The strike is elicited entirely by visual cues so that a prawn presented behind glass is attacked in the usual way. Since the strike fails to seize the prawn, the cuttlefish attacks repeatedly but the rate of striking soon begins to diminish and a typical decremental response curve is obtained. We are here concerned only with the recovery of the response after it has waned: specifically, does the number of strikes in a second presentation period depend on the interval since the first presentation period?

A 'Perspex' cylinder containing seawater and two prawns was placed in a tank about 50 cm from a naive cuttlefish and was then left there for 20 min. During the first 5 min of this period the animal was observed and the number of strikes (*a*) it made was recorded. After a further 15 min, during which the animal was not observed, the tube was removed for an interval and was then replaced for a further 5 min. The number of strikes (*b*) in this second period was recorded and the ratio ($\frac{b}{a} \times 100$) was calculated to give a measure of the percentage recovery of the response for that animal. Animals were tested after eleven different intervals varying from a few minutes to a day. At least twelve animals were tested after each interval; twenty were tested after the intervals 22 min, 30 min, 45 min and 60 min. Each cuttlefish was tested once and then discarded.

As would be expected the response (Fig. 1), which had waned almost completely during the 20 min initial presentation period, showed no recovery when the prawn was offered a few minutes afterwards. More surprising is that even 24 h later the response had only recovered to about 14% of its former value. These animals therefore showed considerable retention. They rarely attacked an animal that is their natural prey, though they attacked crabs presented at the end of the second presentation period: the waning of the response was evidently stimulus-specific and not simply the result of sensory adaptation. Moreover, because crabs were often caught with the tentacles (instead of with the arms alone⁵), it is unlikely that motor fatigue is an important factor in the waning of the response.

It is interesting that the recovery curve is not smooth: the response began to recover and then declined, until at 60 min it had almost disappeared. It then recovered again and by 90 min the response had apparently reached asymptote. That the "dip" in the curve between intervals of 22 min and 90 min is not the result of random fluctuation can be shown by χ^2 analysis of the frequencies of attacks during the first and second presentation, for all these rest intervals. In a 2×5 contingency table $\chi^2 = 34.89$ (d.f. = 4, $P < 0.001$). Furthermore, there is a highly significant difference not only between performance at 60 min and 90 min ($\chi^2 = 33.28$) but also between 60 min and 45 min ($\chi^2 = 13.78$); for both $P < 0.001$ (d.f. = 1). The experiment thus produces the apparently anomalous result that cuttlefish retain more about an event after 1 h than after 20 min.

How can this curve be explained? If it were classical "habituation" Hinde would claim that it results from the interaction of incremental and decremental processes with differing time courses, as, for example, in the recovery of the chaffinch mobbing response^{3,6}. But on present evidence the possibility cannot be excluded that the behaviour is being influenced by negative reinforcement, by pain input from receptors in the tentacles. Several hard strikes of the tentacles on the 'Perspex' may be ample for learning in such fast-learning animals⁷. This experiment, then, may provide an example of a conventional "learning" situation. In such a situation Sanders

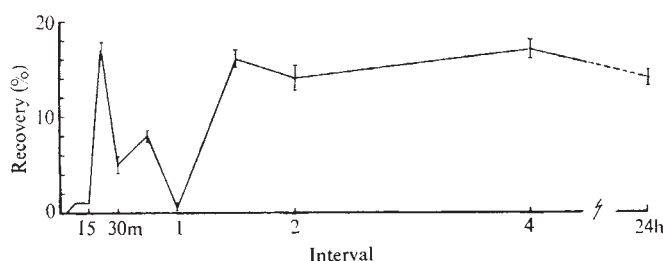


Fig. 1 Percentage recovery as a function of rest interval. Each point is the mean of at least twelve animals: bars indicate s.e.m. Means and s.e. were calculated after arcsin transformation of percentage scores.

and Barlow⁸ have collected data for *Octopus* which show a tripartite retention curve, and their interpretation of this is that there are three memory systems: short, intermediate and long term. It is interesting that the transition from what they call "short-term" to "intermediate" memory occurs at a comparable time to the transition between the two stages of recovery shown here in *Sepia*, which could also be due to the operation of memories of differing time-course.

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Variations in Retention Performance during Long Term Memory Formation

RETENTION performance as a function of time generally follows a typical monotonically decreasing curve¹⁻³, but there are a few reports showing an improvement with time⁴⁻⁹. Monotonic retention curves have been obtained with octopuses for short-term memory (STM)¹⁰ and for long term memory (LTM)¹¹. Here we present a preliminary report of one of a series of experiments using a rapid training procedure which enables us to study changes in retention performance over a period presumed to include the time courses of both STM and LTM.

Experimentally naive *Octopus vulgaris* (150–400 g) which had been in the laboratory for at least 5 days were used when they were attacking and eating crabs readily. These octopuses were trained not to attack a crab (*Carcinus*) in conditions similar to those in which Young¹² has shown that learning is stimulus specific. Training began by placing the crab, tied to a transparent rod, in the home tank at about 50 cm from the octopus. Just before the octopus completed its attack the crab was removed, the octopus was given a 4 V a.c. shock and the crab was immediately replaced. (An attack was judged to have occurred when it was necessary to remove the crab in

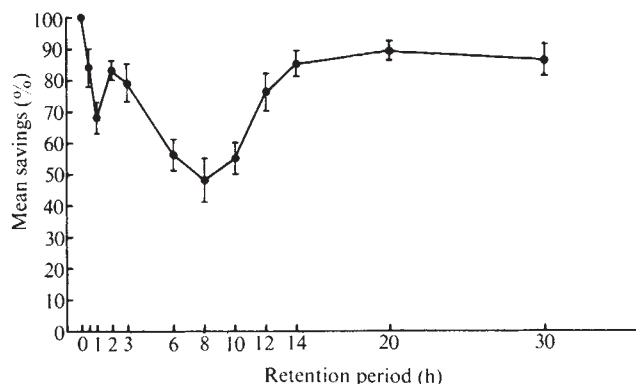


Fig. 1 Retention curve based on the mean percentage savings of octopuses trained and retrained using a 4 V shock to a criterion of no attack on a crab for a period of 1 min. Each point represents the mean scores of six or seven animals. The vertical bars mark one standard error above and below the mean.

order to prevent the octopus from touching it with its arms.) This sequence was repeated until a criterion time of 1 min passed during which there was no attack on the crab. The number of attacks and their latencies were recorded. Different groups of octopuses were retrained by the same procedure after retention intervals of 0.5–30 h. The groups were trained at different times throughout the day so that all retraining occurred during the same period (15.00–19.00 h). The differences between the attacks on training and retraining, expressed as a percentage of the training attacks (% savings), were used as a measure of retention performance. No octopus was retrained more than once.

The mean percentage savings (Fig. 1) decrease rapidly to 68% at 1 h but greater savings are made at 2 h (83%). From 2 h the savings fall steadily to 48% at 8 h only to increase again until at 20 h the octopuses show greater savings on retraining than at any previous time. Retention performance is still good at 30 h. The savings at 1 h are significantly lower than those at 0.5 h ($t=2.00$, $P<0.1$) and at 2 h ($t=2.50$, $P<0.05$). The savings at 8 h are significantly lower than those at 2 h ($t=4.52$, $P<0.01$) and at 14 h ($t=4.59$, $P<0.001$). The octopuses retrained at 1 h made nearly twice as many attacks as those retrained at 2 h while the difference in retraining attacks between 8 and 14 h was three-fold (Table 1). It seems, therefore, that in these conditions retention performance is not monotonically related to the length of the retention interval.

Table 1 Number of Attacks made at Various Intervals after Training

	Retention period (L)											
	0.5	1	2	3	6	8	10	12	14	20	30	
No. per group	6	6	6	7	6	6	7	6	6	7	6	
No. of training attacks: mean	13	15	15	16	15	15	18	18	16	16	14	
s.d.	3.7	4.8	4.4	5.4	2.6	4.4	3.4	4.6	5.6	5.4	3.4	
No. of retraining attacks: mean	1.8	4.7	2.7	3.1	6.3	7.7	8.0	4.5	2.3	2.0	2.0	
s.d.	1.1	1.7	1.5	2.1	1.2	2.9	2.1	2.7	1.5	1.7	1.9	

Several variables may have affected the tendency to attack. All octopuses were last fed with crabs during the afternoon of the day before they were trained. The time between feeding and retraining was therefore about 24 h for all except the 20 and 30 h groups. Thus the principal variations in the numbers of retraining attacks were all obtained at approximately the same hunger level. To carry out retraining during the same part of the day and at the same hunger level, training was given at different times of day. The numbers of training attacks may, therefore, have been influenced both by the hunger level and by possible variations in the daily activity

pattern of the octopuses. The mean training attacks do show some variation (Table 1) but they are not correlated with the mean numbers of retraining attacks ($r=+0.42$, not significant at the 10% level). The differences in training performance would not account for the shape of the retention curve. Changes in activity during the day would not have influenced the retraining attacks as all retraining occurred during the same part of the day. The variation in sea water temperature (16–19°C) was probably not sufficient to have affected the results. We conclude that the variations in the retraining attacks are dependent on the length of the retention interval.

Although there may be other explanations of the shape of this curve we think it most likely that it reflects the state of the available memory. The results would then indicate that the memory for an event is maintained by three processes with different rise and fall times. These may be identified as STM, an intermediate memory (IM) and LTM. According to this hypothesis STM appears during training and declines rapidly, IM appears at about 2 h and declines less rapidly than STM, while LTM appears after approximately 12 h and declines slowly. The STM and IM control behaviour until LTM is available. A similar curvilinear function has been obtained by training *Octopus* with a 10 V and a 3 min criterion. Other combinations of these parameters tended to produce mono-curves with different overall levels of retention performance. This suggests that the present results can be observed only within a limited set of training conditions. A detailed report of these experiments is in preparation.

Previous investigators have found dips in retention curves at 1 h using rats^{4,5}, at 30 min using cuttlefish⁹ and at 2 min (ref. 6) and 30 min (ref. 8) using mice. None of these experiments included systematic tests between 2 and 24 h and so there are, to our knowledge, no reports of the second dip which we find with *Octopus* at 8 h. Evidence for multiple memory storage has depended to a large extent on demonstrations of the selective blocking of memory with electroshock, anaesthetics or antibiotics. On the basis of this evidence Booth¹³ has estimated the time courses of successive physical mechanisms for holding retrievable information during memory consolidation. His expected rise and fall times for the three mechanisms are comparable with those obtained in the present experiment. In view of these observations it seems possible that a system of three memory processes may be universal.

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BOOK REVIEWS

Communities of Sciences

Politics and the Community of Science. By Joseph Haberer. Pp. vi + 337. (Van Nostrand Reinhold: New York and London, February 1971.) £4.50.

Few would disagree with the statement of Dr Haberer that there is a crying need to throw "some light on the political nature of the community of science" and "the political behaviour of its members". Unfortunately, his book, which has been well received in the United States, strikes me as singularly unilluminating.

Professor Haberer is a political scientist who wishes to use history for the purpose of illustrating arguments about how scientists ought to act during social and professional crises. For the author, understanding the past on its own terms is clearly a secondary activity: "political analysis", he asserts on page 218, "does not require exhaustive historical reconstruction". We then must wonder why he devotes several hundred pages to case studies dating back to the seventeenth century. If Haberer is at all serious about the types of evidence he uses, his work must be judged in terms of not only the concision of his concepts but the accuracy of his examples as well.

The author's basic thesis is that scientific communities, when faced with the possibility of severe external sanctions, tend to practise "the politics of prudential acquiescence". According to him, such a tendency derives ultimately from two components of the scientific role, namely lust for power and intellectual arrogance. These norms, in turn, generate a community characterized both by a strong methodological ethic (truth-seeking) and by a weak institutional one. It is the latter quality, in Haberer's view, which generates political "sell-outs" on the part of scientists. Needless to say, this is hardly an attractive picture of scientific life, and we therefore turn with some interest to the data which ostensibly verify it.

From the last three and a half centuries of the history of modern science, the author has selected three incidents to sustain his narrative. The first involves the biographies of Bacon and Descartes (which also serve as his ideal-type projections of the 'Baconian' and 'Cartesian' scientist). Next comes the

experience of leading German scientists during the period of Nazi rule. Finally, we are treated yet again to an exposition of the Oppenheimer affair.

Before analysing the specific events as Haberer discusses them, I think it best to detail a few sins of omission on his part. Surely a treatise on the political behaviour of all Western scientific communities from 1600 to the present which neglects the period 1650–1933, the accumulated experiences of British, French, Italian and Russian researchers, and the activities of all scientists save physicists cannot pretend to be representative, much less exhaustive. The failure to discuss at all the disparate ways in which science became a professional activity in different national settings during the nineteenth century is particularly astonishing, because this process lies at the heart of current battles over the social responsibility of scientists. (Equally astounding to British scientists of interwar vintage will be the author's assertion that "social responsibility" is purely a post-war concern.) I could only wish that Professor Haberer had bothered to explore, however haphazardly, post-Kuhnian developments in the history and sociology of science.

What about his examples? I just cannot see how the relevant scientific communities violated their professional values by refusing to support such court politicians as Bacon and Oppenheimer. Of course this statement sounds ludicrous when applied to Bacon, not only because he was not a scientist but also because there was no scientific community available to protest his downfall. As for the German scientists under Hitler, Haberer's lack of historical perspective leads him to condemn them on the grounds that they lacked the "civic courage" shown by such institutions as the church and the military. Leaving aside our feelings about the scientific community's relationship to Nazism, we should ask whether the author has made the right comparisons. I do not believe he has. The real question is: did the responses of German scientists differ significantly from those of social groups existing in similar institutional contexts, namely other German academics? To the best of my knowledge the answer is negative, which means that we should, in the first instance, look at the operation of the German university system rather

than the universal norms of scientific life. Strangely enough, Fritz Ringer's account of *The Decline of the German Mandarin*, without directly discussing natural scientists, is a far more reliable guide to the actions of the scientific community than Haberer's book.

The author's handling of the Nazi episode really lays bare the poverty and amorphousness of his conceptual structure. What is the community of science? The attempt of Haberer to subsume all scientists and engineers working in academic, governmental, industrial and independent laboratories into a single, transcendent structure flies in the face of atomized, contemporary realities. How representative of the entire "community" are the actions and statements of leading scientists? Here the author assumes a fairly close correlation between leaders and led without ever supporting this notion either theoretically or empirically. What are the values of science which political action might threaten? Here Professor Haberer is at least of three minds. For most of the book, he talks about truth-seeking and independence of judgment. In the final chapter, however, we are told about the advancement of national or humanitarian goals. But he is also led to wonder "whether the community of science has any ultimate or intrinsic values at all—or if any are expressed in the institutional life of the enterprise". How then do we escape from such intellectual and moral bewilderment? At this point, the author comes dangerously close to adopting the "disestablishment of science" recently advocated by Bronowski—a utopian counsel of despair which, to my mind, is as politically irresponsible as it is impracticable.

Given the fact that *Politics and the Community of Science* is a pioneering effort, I realize that I may have been too harsh in my criticism. The reason for this is quite simple. I share the author's conviction—and not a few of his prejudices—that the political role of science in modern society may be the next generation's most critical problem. Apart from a serious and more general commitment to politics as such, we desperately need more hard thinking and judicious inquiry into this subject. I would not like to see such an effort guided by the methods of Professor Haberer.

P. G. WERSKEY

Cells in Charge

Cell Differentiation. Edited by A. Ole Schjeide and Jean de Vellis. Pp. xvi + 606. (Van Nostrand Reinhold: New York and London, February 1971.) £10.

ANY biologist who has the stamina to read this book from cover to cover will emerge tired, but well informed about most of the approaches which are used to study differentiation at the cellular level. But the book describes the state of play, as it were, early in 1968; although the volume is published only this year, I could find no references to work reported in the literature after 1968, comparatively few references to work reported in that year, and citations to "recent reviews" proved to refer to articles published in 1967 or even 1966. But it is a mark that the book is well written and put together that comparatively little of what it says would now be regarded as misleading; it is merely a shame that the further experiments performed in the past year or so, which in some instances confirm suggestions made by the authors, are not presented to buttress these conclusions.

The first section of the book, entitled "General Concepts", comprises three chapters, the first of which, written by the two editors, should be compulsory reading for all cell biologists; this brief discussion is notable for the sense of perspective which it conveys of cell biology in general and of the succeeding chapters of this book in particular. But the two chapters which follow are perhaps a little too theoretical to fit well with the other, experimentally orientated chapters, and are unlikely to appeal to the graduate student level audience at which the book seems to be aimed.

The second and fourth sections are probably the most valuable. The second section, molecular biology, starts with a chapter which is very largely a review of the classical molecular biology of bacteria; as such this is not particularly valuable, for there are many other competent treatments of this subject. Although the editors no doubt felt that it would be best for this book to be complete in itself, I doubt the usefulness of yet another review of the evidence that DNA is the genetic material, to cite one example. A volume written at this level would do better to take for granted the classic studies of nucleic acid and protein synthesis and to turn directly to its topic. But, that said, the remainder of this section, which discusses the transcription of RNA, the cell cycle, nucleocytoplasmic interactions and membrane functions, is well written, reasonably thorough, and is bound to be useful for reference.

The succeeding section, on biochemistry and metabolism, which is concerned with topics such as the ageing of cells and changes in enzyme and other protein activities during differentiation, is perhaps too didactic to be more than a sidestream in cell differentiation.

The final section, which makes up half of the book, is concerned with particular systems which are being used to study differentiation. The first two chapters concern the differentiation of the cell as such, that is to say, the synthesis of structures such as polyosomes and the endoplasmic reticulum. The chapters which then discuss the details of several individual systems are all useful as reference works describing the situation as it was early in 1968.

A notable omission from the book, which is perhaps not surprising in view of its age, is a discussion of cell hybridization and the uses to which this versatile technique may be put. If a second edition is prepared, the editors would do well to perform some judicious pruning, especially on the two more speculative chapters of the first section and of the review of molecular biology. A book of this nature is at its most useful when recently written, so it is to be hoped that such delays in production will not be repeated. And it is fair to add that a book as large and complex as this one is only as useful as its index, which is, alas, so perfunctory as to be useless.

BENJAMIN LEWIN

Introducing Microbes

Micro-organisms: Function, Form and Environment. Edited by Lilian E. Hawker and Alan H. Linton. Pp. xxii + 727. (Arnold: London, January 1971.) £6.

IN an effort to provide an up to date undergraduate text in microbiology, Professor Lilian Hawker and Dr Alan Linton have enlisted the aid of twenty of their colleagues, each contributing one or more specialist chapters or sections. The aim has been to survey the whole field of microbiology so as to make it readily understandable by first year university students. Only a relatively small part of the book is devoted to strictly biochemical and genetical topics, an imbalance which has been made deliberately by the editors, who feel that this material is covered extensively in the existing textbooks. Discussions of growth and viability are followed by the two largest sections of the book, which deal with microbial structure, life cycles and classification and with microbial ecology. The final few chapters are devoted to selected aspects of economic microbiology.

The greatest difficulties confronting editors of a text produced in this way are those of achieving conformity of style, and balance and integrity of material. It is clear from the cross-referencing that the editors have been at pains to try to interrelate the contributions of the various authors but, nevertheless, a disappointment of the book for me was that the integration was not more fully realized. The treatment of growth physiology is the best to be found in any text at this level and, in particular, continuous cultivation is given much more than the usual nominal treatment. It is unfortunate therefore that reference to the application of steady state systems was not made by other contributors, who missed an ideal opportunity of emphasizing the dynamic nature of the chemistry, structure and functioning of microorganisms. In this context a rearrangement of chapters might have helped. For example, discussion of microbial nutrition and the effects of environmental parameters on growth could have been more effective after the reader had been introduced to chemostasis.

All the major groups of the Protista are covered comprehensively and the discussion has been based firmly on classical taxonomies. The latter approach does not make very compulsive reading but, at the same time, the student will be able to discover readily the breadth of his subject and find his way around the diverse groups of microorganisms. This section concludes with a useful account of numerical taxonomy but surely the reader deserves an assessment of the success of this rediscovered system of classifying, how it works out in practice and how widely it has been applied, without having to resort to the specialist primary literature. My only quibble with the otherwise interesting discussion of economic microbiology is that it tends to be too descriptive. The reader will find little or nothing on process design, applied genetics, new fermentation methods or cost analysis, all of which are of crucial importance to the industrial microbiologist.

The few reservations which I have about this book are offset by its general excellence, and certainly it will figure on my list of recommended first year texts. Microbiology is presented in the widest possible sense and the contributions from specialist authors have provided some fascinating and stimulating reading. The illustrations have been chosen with great care and are of the highest standard; they add much to the reader's comprehension and pleasure. Indeed, the whole production has been meticulous, and remarkably few printing or other errors have impinged on my eye. Specific bibliographies have,

on the whole, been well selected and, most important in my view, the book is supplied with a good index. In conclusion this is a most welcome addition to teaching texts in microbiology and even if the price is high it is, alas, not now unusual for books of this kind.

ALAN T. BULL

Bug Killers

Naturally Occurring Insecticides. By Martin Jacobson and D. G. Crosby. Pp. xii + 585. (Dekker: New York, January 1971.) \$33.75.

THE increasing apprehension over the use and persistence of synthetic insecticides on the one hand, and the build-up of insect resistance to many of these compounds on the other, has led scientists to search for other methods of chemical control of harmful insects. Fortunately there were already leads to be followed in existing biological control, and this book sets out to review the chemistry, biochemistry, toxicology and biology of the various compounds involved. The book is presented in three sections, depending on the source of the insecticides: botanical, insect-derived, and bacterial and fungal. Each chapter is written by a world authority on the subject although naturally there is considerable variation in the thoroughness of the treatment, and each has its own collection of references to the original literature.

The first section comprises chapters on the pyrethroids, rotenone and the rotenoids, nicotine and other tobacco alkaloids, the unsaturated isobutyramides and finally minor insecticides of plant origin. Each of these chapters contains detailed chemical treatment of the compounds involved as well as descriptions of application and effectiveness of treatment. The second section deals with arthropod venoms, and secretions and cantharidin, the juvenile hormones, and the ecdysones. Unfortunately the chapter on juvenile hormones, unlike most of the others, contains little chemical information on determination of structure and nothing on the numerous synthetic approaches to this important compound. In the final section the use of *Bacillus thuringiensis* as a microbial insecticide and a description of the chemistry and application of the destruxins and pericidins is given. The book is the first collection of such material and will be an important reference volume for the many scientists working in this large area. Unfortunately there are very few references to work published within the past two years which, therefore, excludes reference to much important work in this rapidly expanding field.

A. W. JOHNSON

High Speed Introduction

An Introduction to Ultracentrifugation. By T. J. Bowen and A. J. Rowe. Pp. xviii + 171. (Wiley-Interscience: London and New York, December 1970.) £2.50.

THE ultracentrifuge is now so fundamental to the study of proteins and nucleic acids that an early introduction to the machine must form an essential part of the training of the research worker in these fields. And while there are several excellent reviews and monographs devoted to its use, an elementary but comprehensive text has so far been lacking. This book is intended to fill this gap; as the preface emphasizes, the treatment is purposely elementary, and the mathematical content approaches the minimal. Judgment on the book must accordingly be made within this frame of reference.

In the introductory chapter, the elementary theory of sedimentation velocity and its relation to molecular weight are outlined, and some of the early and now classical results are surveyed. The characteristics of the more important modern analytical instruments are then summarized; one wishes, perhaps, that the authors had been less guarded in their reference to the costs of maintenance and spare parts. Optical systems are described clearly: the diagrams illustrating the relation between the type of cell and the patterns it provides will be particularly useful to the beginner.

A chapter on the determination of sedimentation and diffusion coefficients, and a discussion of their significance, is followed by one devoted to the Lamm equation. Even allowing for the author's purpose, the treatment seems oversimplified: phrases such as "the velocity of the solute molecules" are to be found, and equilibrium is referred to as a "steady state". The treatment of diffusion is in terms of opposing osmotic and diffusion pressures (surely hypothetical) and the force-friction concept; to me, the phenomenological treatment of Gosting and others seems preferable, in its adherence to strictly operational concepts. Nevertheless, the essential significance of the transport coefficients is adequately described, and their relationship to experimental measurements established. The treatment of the continuity equation is detailed, but the attempt to avoid partial differentials leads to a rather cumbersome form for the important equations: once again, the explicit formulation of a flow would lead to a simpler result.

Sedimentation equilibrium is now firmly established as a principle of major importance in ultracentrifuge work, and the value of a book in this field must depend significantly on the clarity with which the essential concepts are described. It is sufficient of a comment

that the equations of equilibrium are described here only in the context of zero flow: it is regrettable that the instructive comparison between equilibrium and the Archibald principle is thereby lost.

In the chapter on the determination of molecular weights, the most important of the many methods now in use are described. Due prominence is given to the high speed method of Yphantis but there is no guidance on the fundamental question of speed selection, nor is the simple expression stated for the time taken to attain equilibrium.

The chapter on the determination of molecular conformation provides a useful summary of procedures based on hydrodynamic models and this leads naturally to a discussion of the intrinsic viscosity and how it is determined. One might question the necessity for some of the detail in this section; Tanford's explicit equations relating the sedimentation coefficient and the intrinsic viscosity to the molecular weight of proteins as random coils could usefully have been included.

In the analysis of mixtures by sedimentation velocity, two complications are well recognized, if not always allowed for: these are the Johnston-Ogston effect and the more specific molecular associations associated with the name of Gilbert. Both these topics are discussed, and the essential precautions set out.

Dr Bowen's final chapter is concerned with preparative ultracentrifugation. It describes how preparative runs can be planned intelligently, and collects together much material that is widely scattered in the literature and in makers' manuals. The relative advantages of different types of rotor are assessed, and many quantitative comparisons are made: the section should be of particular value to people concerned with zonal separations.

The last two chapters, by Dr Rowe, are concerned with applications and some commonly encountered problems. Appropriately, emphasis is given to the problem of assessing the degree of homogeneity of substances examined in the analytical ultracentrifuge. As the author states, much of the evidence concerning homogeneity presented in the literature is not wholly convincing. The standard methods based on velocity measurements are described; in those based on sedimentation equilibrium, reliance is placed heavily on the very recent methods evolved by the author himself. It would have been helpful to have included the informative examples of Fujita and Williams. Some good examples of typical conformational changes in proteins and nucleic acids are quoted.

In the section on problems, some

typical questions are answered, and ways of overcoming difficulties suggested. There is much sense in this, although the occasionally flippant style may not be to every reader's taste. Some numerical problems are also included, with answers.

Although most of the errors are relatively trivial, some could mislead the inexperienced worker. Thus, on page 46, the suggested method for determining diffusion coefficients will not give a straight line plot; the defining equation for $\bar{v}$ (page 56) has an unnecessary subscript which obscures the significance of the differential; the initial concentration in the example of the Yphantis method (page 68) must be wrong by at least an order of magnitude. Most seriously, in the section on the Archibald method, the concentration at the meniscus is erroneously identified (equation 6.23) with the difference between the initial and plateau-region concentrations. Although this mistake is repeated in the example, the meniscus concentration is correct. Equations 6.26 and 6.27 are incorrect. In equation 7.20 the critical subscript to the partial differential is undefined. On pages 152 and 153, the symbol "l" appears where "p" is intended; once again, the critical subscript is omitted. Equation 10.12 is incorrect: the quantity ϕ' defined by Casassa and Eisenberg should replace $(\partial/\partial c)$.

In spite of these errors and omissions, and some deficiencies in editing, I feel that this book's purpose has been achieved. The student who has read it will be in a position both to perform simple experiments and to understand the more rigorous approach of the primary sources, to which he will be led by generally excellent documentation. At its modest price, the book is good value.

J. M. CREETH

Electron Optics

Electron Optics. By O. Klemperer. Third edition in collaboration with M. E. Barnett. (Cambridge Monographs on Physics.) Pp. ix+506. (Cambridge University: London, January 1971.) £9.

THIS third edition is probably the best and most comprehensive introduction to electron optics currently available in English. It reflects the considerable developments that have taken place since the previous edition was written in 1953. In spite of the addition of a considerable amount of new work, the size of the book has increased by only seven per cent, the result of some vigorous pruning and rearrangement of material.

Descriptions of miscellaneous standard electron optical devices have been replaced by topics of a more funda-

mental character. The result is a work that reveals, with much greater clarity than before, the essentials of the subject. The authors have taken special pains to anticipate and alleviate the difficulties often experienced by students of electron optics. Thus, a single consistent set of practical units is used throughout. The authors perhaps carry discretion too far in not mentioning what the system is and also in expunging from this edition any mention of alternative systems including SI. This together with the indiscriminate use of the symbol μ (surely a printer's error?) both for the permeability of free space and that of ferromagnetic materials may temporarily confuse the unwary. Strangely enough, the word permeability does not occur in the index, probably the weakest part of the present volume. The general treatment of the subject, however, is highly praiseworthy. Important concepts such as vector potential are first defined mathematically and then interpreted physically.

Likewise, in order to direct attention to the essential steps in the argument, simple, even clumsy, mathematical symbols have been preferred to more elegant but elusive shorthand notation. The mathematical treatment is designed to appeal to experimental physicists rather than to mathematicians and is closely interwoven with experimental results and illustrative material.

All important topics are covered, some in greater detail than others. An extensive and well arranged bibliography directs the curious to the appropriate source material. The book can, therefore, be strongly recommended to students, young and old, of electron optics and indeed as a work of reference to all who are engaged in research and development in electron optics.

T. MULVEY

Introducing Information

Introduction to Information Science. Compiled and edited by Tefko Saracevic. Pp. xxiv+751. (Bowker: New York and London, 1970.) \$27.50.

INFORMATION science is a name that might be applied to many areas of study related to information theory in the Shannon-Weaver sense, as well as to information processing by computer. In recent years, several university post-graduate schools have coupled the words with librarianship, to imply what has also been called documentation, but the content of this teaching is still principally the art and practice of organizing documentary records of all kinds, and it might seem presumptuous to claim that this could be called a science.

This book aims to demonstrate that

beyond the pragmatic arts of information organization, there is emerging an academic discipline that is objectively concerned with an area of human activity—communication processes particularly as they are mediated by information systems. Information is generated from a myriad of sources, often in documentary form, and it is sought by a myriad of users. The two streams merge in a complex network of information systems. The study of the structure, objectives, functions, properties, behaviour and performance of information systems and of their individual components (human or inorganic) is the emergent field of "information science".

There is as yet no formal textbook of the subject. The book under review is a collection of sixty-six papers by leading investigators, published mostly during the past decade. The papers are almost exclusively theoretical or experimental, and do not include pragmatic descriptions of the development or operation of information systems. The claim that the book "introduces" information science is justified by skilful editorial organization and comment that aims to display the content of and interrelationships within the science. As structured by Saracevic, the field covered includes communication processes (interrelationships exhibited by the documentary record and the behaviour of information users), the structure and attributes of information systems and procedures, and the evaluation of systems. The final paper is an attempt to outline a general theory of communication, by W. Goffman.

Information science has become quantitative and experimental. There is a good deal of mathematical modelling in these papers, and the development and use of system performance measures. There is also much descriptive analysis of the structure of knowledge, of information use and so on. That a recognizably distinct discipline has emerged is clearly demonstrated, though it is also clear that it is still a very young science. Its object of study is a social relationship (communication) achieved through physical media (such as books or computers), and this complexity accounts for the peculiar difficulty of placing it in any traditional "system of the sciences".

The selections brought together by Saracevic do represent the most thoughtful and stimulating work that has been done in this new field, though it is of course easy to regret the absence of significant papers. The book should indeed serve to "introduce information science" to workers in related fields, and be a useful source for students. Unlike many books of "readings", it makes a real contribution to the development of its subject.

B. C. VICKERY

CORRESPONDENCE

Aid for Bengal

SIR,—I feel that the letter by K. C. Tripathi (*Nature*, 232, 69; 1971) does not do due justice to the splendid humane effort that India has exerted in the past (and present) Bengal crisis. It is all too easy, sitting in an economically and socially cosy island some 5,000 miles from Bengal, to decry the efforts of the Indian authorities in their troubles. K. C. Tripathi should try to visualize 6 million Pakistani refugees suffering from disease

and starvation swarming across the Oxfordshire border and having only the resources of the Oxfordshire County Council to organize the relief aid, to appreciate just how stupendous would be the task of helping the refugees.

It is a fairly simple matter for the "developed" countries to send a few spare planes loaded with surplus stores and medicines and then to expect someone else to do the dirty work of organizing the distribution of the relief aid dumped on their doorstep.

The people of India have made a huge sacrifice in relation to their gross national product and their generosity and humanity should be laudified and not decried.

Yours faithfully,

P. J. S. BROOKS

*Cottage by the Ford,
Mill Lane,
Middle Barton,
Oxfordshire*

Obituaries

Professor I. Vincke

IGNACE VINCKE, the eminent Belgian malariologist, died in Antwerp on March 13 at the age of sixty-five. He was born in Bruges where the family had long been established; his grandfather was Burgomaster and his father carried on the traditional occupation of growing bay trees for princely estates in Germany and the Low Countries. Ignace himself inherited "green fingers" and as a medical student he was awarded a Royal Medal for his discovery of a method for the sterile cultivation of orchid seeds. He graduated in medicine at the University of Ghent and, after taking the course in tropical medicine at the Institut Prince Léopold in Antwerp, embarked in 1931 for the Belgian Congo.

The starting point of Vincke's career was the study of the African anopheline mosquitoes, amongst which was *Anopheles durenii*, as the assistant of Albert Duren. He discovered sporozoites of a malaria parasite in this mosquito and for 5 years sought in vain the vertebrate host, which was clearly not man as the infected specimens were caught in uninhabited forest. In 1948 he traced the parasite to an arboreal rodent, *Grammomys surdaster*; he inoculated the blood into mice and rats and isolated a strain (*Plasmodium berghei*) which quickly became adapted as a model for the study of malaria in small laboratory animals. Within a year, the parasite was distributed throughout the world, and proved so useful that more than a thousand scientific papers have been written about it.

Strangely enough, Vincke's own interests veered from this discovery to the more pedestrian though more humanitarian pursuits of malaria control and eradication. But he continued his observations on the blood parasites of sylvatic rodents, and isolated another malaria

parasite which was named *P. vinckei* in his honour, and a piroplasm which he named *Babesia rodhaini* in honour of his famous teacher, and both these organisms have been widely used in parasitological research. He worked on these problems, first in the Congo, then on various World Health Organization assignments and finally in the Department of Malariology in the Tropical Institute in Antwerp. His entomological interests remained and, as a result of his deep knowledge of the bionomics of mosquitoes, he was able both to apply DDT with intelligence in the rural zones of tropical Africa to destroy mosquitoes and to establish highly successful colonies in his insectaries in the Institut pour les Recherches Scientifiques en Afrique Centrale, and in Antwerp.

Vincke had a curious, unconventional personality. He loathed officialdom and once said, after a particularly frustrating period in the Congo, that "the only good DMS is a dead DMS"! He was quiet though mischievous (the late Professor Jacques Schwetz, also of the Congo Service, described him as a "méchant enfant"), charming and yet withdrawn, but with above all a great sense of modesty. The grand maître of Belgian tropical medicine, Jérôme Rodhain, appreciated the unusual talents of his pupil and foresaw a great future for him. His eminence was recognized with various awards, including the Prix Broden from Belgium and the Prix Carlos Chagas of Brazil, and by his elevation as Commander in the Order of Léopold.

Academician V. V. Parin

ACADEMICIAN Vasilii Vasilevich Parin, one of the leading physiologists and experts on space medicine in the Soviet Union, died on June 15, 1971.

Parin was born in 1903, in Kazan'. He graduated in 1925 from the Medical Faculty of Perm University and from 1930 to 1947 he held the post of head of the Department of Physiology at institutes in Perm, Sverdlovsk and Moscow. From 1942 to 1945 he was Deputy People's Commissar of Health of the USSR, and in 1944 he became a founder member and first Academic Secretary of the Academy of Medical Sciences of the USSR.

His academic career was interrupted in 1947, when he was imprisoned in a labour camp. After his release in 1954 he became head of the Physiology Laboratory of the Institute of Therapy of the Academy of Medical Sciences. Later appointments included head of the Faculty of Clinical and Experimental Physiology of the Central Postgraduate Medical Institute, director of the Institute of Normal and Pathological Physiology of the Academy of Medical Sciences, director of the Institute of Medico-Biological Problems of the Ministry of Health of the USSR, director of the Laboratory of the Control of Functions in Human and Animal Organisms of the Academy of Sciences of the USSR, and deputy academic secretary of the Department of Physiology of the Academy of Sciences. He was closely associated with the Soviet space programme from the earliest launches of rockets bearing test animals, and is understood to have been one of the principal organizers of the medical and biological experiments in the space programme.

He was on the editorial board of several physiological and medical journals and he published over 200 scientific and popular works on the regulation of blood circulation and space physiology and cardiology. He was also a member of Soviet delegations to a number of international conferences, a member of the International Astronautics Academy and an honorary member of the Rumanian

Academy of Sciences and the Purkinje Czechoslovak Medical Society. The awards he received included the Order of Lenin and the Order of the Red Banner of Labour.

Announcements

University News

Dr J. B. Lloyd, University College, Cardiff, has been appointed research professor in biochemistry in the University of Keele.

Dr Peter Harris has been awarded a personal professorship in the Department of Earth Sciences, University of Leeds.

The title of professor of physics has been conferred on **Dr P. V. March** in respect of his post at Westfield College, University of London, and that of professor of pathology has been conferred on **Dr T. Symington** in respect of his post at the Institute of Cancer Research, Royal Cancer Hospital.

Miscellaneous

The first **Florey fellowship** has been awarded to **Dr G. G. MacPherson**, MRC junior research fellow at the Sir William Dunn School of Pathology, University of Oxford, to enable him to work for two years on the physiology of the lymphatic system and its role in transplantation immunity, at the John Curtin School of Medical Research, Australian National University.

Bruno Mendel travelling fellowships have been awarded by the Council of the Royal Society to **Mr C. M. Colley**, University of Cambridge, and to **Miss M. Small**, Weizmann Institute of Science. Mr Colley will study the interactions between lipids and proteins in cell membranes at the State University, Utrecht, and Miss Small will work at the Chester Beatty Research Institute on the interaction between a

lymphocyte activator from thymus and its possible target cells.

The **Fondación Viviana Luckhaus international prize** will be awarded in 1972 for a paper describing original research work related to blood platelets and/or their relationship to thrombosis and blood vessels. The prize will consist of an award of 18,188 Lay (3,000 dollars), a medal and a ticket and expenses for a one-week trip to Buenos Aires, and is open to investigators of all nationalities. Further information can be obtained from **Dr Jorge Buraschi**, Fundación Viviana Luckhaus, Hospital Juan Fernandez, Cerviño 3356, Buenos Aires, Argentina.

International Meetings

September 11–26, **Swiss National Autumn Fair**, Lausanne (Association des Intérêts de Lausanne, Rue Chancrau 3, Lausanne, Switzerland).

September 28–October 2, **Europrotection**, Paris (Europrotection, 8 rue de la Michodière, 75 Paris 2e, France).

September 29–October 1, **Research and Development**, The Hague (Financial Times Conference Department, 338 Strand, London WC2R 0LT).

October 4–6, **Turbulence in Liquids**, Rolla (John Short, Continuing Education, University of Missouri-Rolla, Rolla, Missouri 65401, USA).

November 24, **Computer aided Microscopy**, London (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1).

Reports and Publications

not included in the monthly Books Supplement

Other Countries

Zoology Publications from Victoria University of Wellington, No. 53: Some Improvements in Zoological Microtechnique for Electron Microscopy. By H. W. Johnston and M. N. Loper. Pp. 7 (2 plates). (Wellington: Victoria University of Wellington, 1970.) [224]

Smithsonian Contributions to the Earth Sciences, No. 5: The Ellende, Mexico, Meteorite Shower. By Royal S. Clarke, Jr., et al. Pp. iv + 53. (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) \$1.25. [234]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 186: Devonian Stratigraphy of Northeastern British Columbia. By G. C. Taylor and W. S. MacKenzie. Pp. 62. \$1.50. Paper 70-2, Part B: Geological Survey of Canada—Radiocarbon Dates X. By J. A. Lowdon, R. Wilmet and W. Blake, Jr. Pp. 472–485. \$0.75. Paper 70-13: Lower and Middle Devonian Stromatopora from Northwestern Canada. By C. W. Stearn and P. N. Mehrotra. Pp. 32 + 6 plates. Paper 70-62: List of Translations in the Library of the Geological Survey of Canada, No. 1. Compiled by L. Swirsky. Pp. 25. \$0.75. Index of Publications, Geological Survey of Canada, 1959–1969. Pp. 123. \$2. (Ottawa: Queen's Printer, 1970 and 1971.) [234]

US Department of the Interior: Geological Survey, Bulletin 1312-0: Mineralogy and Geochemistry of Some Belt Rocks, Montana and Idaho. By J. E. Harrison and D. J. Grimes. Pp. iv + 49. \$0.35. Professional Paper 534: Mississippian Rugose Corals Peratrovich Formation, West Coast, Prince of Wales Island, Southeastern Alaska. By Augustus K. Armstrong. Pp. iv + 44 + 13 plates. \$1. (Washington, DC: Government Printing Office, 1970.) [234]

National Museums of Canada—National Museum of Man. Publications in Archaeology, No. 1: A Review of Alberta Archaeology to 1964. By Richard G. Forbis. Pp. ix + 49. \$1.50. Publications in Ethnology, No. 1: t'a: t'a: qsa: A Practical Orthography for Nootka. By A. Thomas and E. Y. Arima. Pp. vi + 35. \$1.50. No. 2: The Girl Who Married the Bear. By C. McClellan. Pp. x + 58. \$1.50. Publications in History, No. 1: Two Centuries of Ceramics in the Richelieu Valley. By Helen H. Lambart. Pp. vi + 34. \$1. No. 2: The Rivers of the Pottery. By Helen H. Lambart. Pp. viii + 30. \$1. No. 3: Plaisance. By John Humphreys. Pp. viii + 24. \$0.75. No. 4: Bibliography of the Material Culture of New France. By Barbara Alexandrin and Robert Bothwell. Pp. vii + 32. \$1. National Museums of Canada—National Museum of Natural Sciences. Publications in Palaeontology, No. 3: A New Teleost from the Oldman Formation (Cretaceous) of Alberta. By David Bardack. Pp. vii + 8. \$0.50. No. 4: Comments on Dinosaurian Preservation in the Cretaceous of Alberta and Wyoming. By Charles M. Sternberg. Pp. vi + 9. \$0.50. No. 5: Biostratigraphy and Palaeontology of a Sangamon Deposit at Fort Qu'Appelle, Saskatchewan. By E. Khan. Pp. viii + 82. \$2. No. 6: A Fossil Ray, Possibly *Myledaphus* (Elasmobranchii: Batoidea) from the Late Cretaceous Oldman Formation of Western Canada. By Wann Langston, Jr. Pp. vii + 15. \$0.50. Publications in Zoology, No. 3: Shore and Freshwater Fish Collections from Newfoundland. By W. H. Van Vliet. Pp. vi + 30. \$1. (Ottawa: National Museums of Canada, 1970.) [234]

Novos Taxa Entomologicos, No. 84 (November 1970): On Some New Genera and Species of Armoured Scale Insects (*Homoptera: Diaspididae*) from Southern Africa. By J. Munting. Pp. 14. (Lourenço Marques: Institut de Investigação Científica de Mocimboa, 1970.) [264]

Institut Royal Météorologique de Belgique. Publications, Série A, No. 71: Distribution du Rayonnement Solaire en Belgique. Par R. Dogniaux. (Bruxelles: Institut Royal Météorologique de Belgique, 1971.) [264]

The Physics Problems of Reactor Shielding—Report of a Joint ENEA/IAEA Specialist Meeting, Paris, December 1970. Pp. 175. (Paris: Organization for Economic Co-operation and Development, 1971.) 23 francs; £1.75; \$5. [264]

UNISIST—Synopsis of the Feasibility Study on a World Science Information System. By the United Nations Educational, Scientific and Cultural Organization and the International Council of Scientific Unions. Pp. 92. (Paris: Unesco, 1971.) 16 francs; \$4; £1.20. [274]

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